

Immunity and Hypersensitivity Relationship to Disease in Man

Report of the Ninth M & R

**P E D I A T R I C
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Issued by M & R Laboratories, Columbus 16 Ohio

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Megaloblastic Anemia	May 1951
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Carbohydrate Metabolism	April 1952
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Pulmonary Hyaline Membranes	January 1953
Potassium Metabolism	April 1953
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Renal Function in Infants and Children	December 1953
Vitamin B ₁₂ in Human Nutrition	March 1954
Fat Metabolism	October 1954
Diabetes Mellitus in Infants and Children	January 1955

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The Ninth H & R Pediatric Research Conference, a symposium on immunity and hypersensitivity relationship to disease in man, was held under the auspices of the Department of Pediatrics of Stanford University School of Medicine, at Lane Hall, San Francisco, California; on October 30 and 31 1958.

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Basic Concepts

Introduction

DR. SIDNEY RAFFEL. Effective studies of the nature of immunologic phenomena began to blossom for the second time some fifteen years ago. For such various disciplines as physical chemistry, nuclear physics, biochemistry, pharmacology, and endocrinology had provided the ultracentrifuge, electrophoretic apparatus, tagged atoms, purified endocrine secretions and specific cellular poisons. Many concepts previously not studied for lack of suitable methods were explored through these new techniques. Thus contributions from the physical sciences applied to the biological and clinical sciences have produced progress in the understanding of immunology.

Immunologic investigations follow two main paths of interest. The first is concerned with infectious disease and the second with studies of hypersensitivity and of immunologic disease, i.e., disease predicated upon the destruction of an individual cell by the action of antibodies. Both areas of interest are closely related because the same considerations of specificity of antigenicity and of host response apply in acquired immunity to parasites and toxins, in antibody formation by the hypersensitive subject, and in the manufacture of autoantibodies against tissue components, the acquisition of antibodies transplacentally or by transfusion. Knowledge of such phenomena acquired during the past ten or fifteen years constitutes the basis for the present symposium.

The immunologic response of the host has been found to differ qualitatively as well as quantitatively depending upon the chemical nature of an antigen, the route by which this gains access to the tissues, the intensity and duration of exposure of the tissues to the antigen, and the species of the responding animal. Thus administration of rabbit serum albumin to the horse results in antibody with an electrophoretic mobility between beta and gamma globulin; this antibody will not flocculate with antigen except in a very narrow range of mutual concentrations. On the other hand, rabbit

serum globulin administered to a horse evokes the formation of antibody of high molecular weight, about 900,000, with a very broad reactive ability with its antigen. Analogous information is available for various antibody responses in the human being.

Studies of infectious disease have also clarified our understanding of the natures of immunizing substances. It was once thought that acquired resistance to all bacteria might depend upon antigenic components of the capsule or cell surface. The fallacy of such a generalization is apparent from the example of anthrax bacillus, for antibodies formed against the highly antigenic capsular substance of this bacterium do not confer immunity. The development of antibodies by the host in this case reflects simply a conformity to the physiologic law that the animal body responds in this way to substances foreign to the tissues. There is not inherent in this response a protective function.

In the field of diseases of hypersensitivity an awareness of the abilities of simple chemical substances to become antigenic through association with tissue protein has increased our understanding of the occurrence of these affections. Tissue changes which take place in experimental animals undergoing hypersensitive reactions have been compared with alterations observed in human subjects in some disease states, and this comparison serves as a basis for the classification of rheumatic fever as a disease of hypersensitivity.

Diseases of immunologic origin such as erythroblastosis fetalis and certain instances of hemolytic anemia and thrombocytopenia purpura define circumstances in which the maternal organism or the affected subject himself seems bent upon fetal or self-destruction.

During the course of this symposium several facets of this complex subject of immunity and hypersensitivity will be more fully explained.

Cellular Sources of Antibodies

Nature of antibody

DR. WILLIAM E. ENRICH Since the turn of the century the globulin nature of antibody has been recognized. Recently it has been shown by the electrophoretic technique that antibody migrates with or close to gamma globulin, although not all gamma globulin is antibody. The gamma globulin or proteins similar to gamma globulin produced in cases of multiple myeloma are non-antibody protein. Hyperimmunization of rabbits leads to the replacement of

all non-antibody globulin by antibody globulin and, in contrast, patients with congenital agammaglobulinemia lack the ability to form antibody. In a discussion of the cellular sources of antibody non-antibody gamma globulin must be considered.

The plasma cell as source

The cells commonly considered as sources of antibody and gamma globulin are the reticuloendothelial cells, the lymphocytes, and the plasma cells. Our current studies point to the plasma cell as the source of antibody and gamma globulin. Other investigators favor the lymphocyte, or classify the plasma cell as a functional stage of reticuloendothelial cell or lymphocyte development.

Reticuloendothelial cells or macrophages are phagocytic and arise from nonphagocytic undifferentiated mesenchymal cells. The macrophage may occur either as a fixed reticuloendothelial cell, a free monocyte in the blood and lymph, or a histiocyte in tissue. A fixed reticuloendothelial cell develops from a fixed mesenchymal cell. Becoming migratory in tissue it is classified a histiocyte. Circulating monocytes arise from fixed undifferentiated mesenchymal cells passing through a blast form. The monocyte migrating into tissue, or grown in tissue culture may develop into a histiocyte indistinguishable from those derived from fixed reticuloendothelial cells.

The view that mature macrophages or lymphocytes can change into plasma cells is at variance with our knowledge of ontogenesis. Lymphocytes and plasma cells arise from undifferentiated mesenchymal cells passing through blast forms. Lymphocytes develop from lymph blasts and are small pyknotic cells which seem to disintegrate within a few days, in contrast to the macrophage, and are found reduced in number in the circulating blood following the release or administration of adrenal cortical steroids. Plasmoblasts undergo differentiation and maturation, to disintegrate within one week.

Clinical evidence

On clinical grounds there is no support for the concept that either antibody or gamma globulin arises from the lymphocyte or reticuloendothelial cell. Hyperglobulinemia is an unusual finding in cases of lymphocytic or monocytic leukemia or reticuloendothelial cell sarcoma. Experimentally no one has isolated antibody from the lymphocytes of the thymus cortex or the circulating blood.

Macrophages harvested from the peritoneal cavity do not yield antibody

These results contrast sharply with the clinical and experimental observations made on plasma cells. With plasma cell proliferation in patient, experimental animal, or tissue culture, a rise in antibody formation or non-antibody gamma globulin is noted. The highest levels of serum gamma globulin are to be found in patients with multiple myeloma

Cytologic evidence

Cytologically the plasma cell, in contrast to the reticuloendothelial cell or the lymphocyte resembles a cell actively engaged

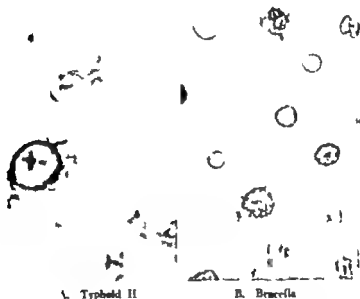


Figure 1. Agglutination of bacteria on the cell membrane of plasma cell. 1000. Reprinted from the Proceedings of the Society of Experimental Biology and Medicine 74:731 (1950)

in protein synthesis. The cytoplasm is rich in ribonucleic acid and the electronmicroscope reveals structures found only in cells actively elaborating protein. Russell bodies, believed to represent retained protein secretions, are frequently found in plasma cells. With phase microscopy dark droplets believed to be organs of protein synthesis, if not synthesized protein, have been described within the

cytoplasm of the plasma cell. These droplets are not found in the lymphocyte or reticuloendothelial cell.

Immunologic evidence

Immunologic evidence is available in support of the concept that the plasma cell elaborates antibody. Plasma cells isolated from an antibody forming lymph node will agglutinate bacteria and dissolved antigen on the cell membrane. Macrophages and lymphocytes do not manifest this activity (figure 1). A globulin has been successfully extracted from plasma cells isolated from a case of multiple myeloma, having the same electrophoretic mobility as the serum globulin producing the hyperglobulinemia of the patient.

These observations lend support to a genetic theory of antibody formation, by which the antigen does not act on gamma globulin, as suggested by Pauling, or on the cytoplasm of the antibody forming cells, as postulated by Burnett, but rather by which antigen functions as an organizer inducing undifferentiated mesenchymal cells—the plasmablast to form a specific plasma cell equipped to synthesize a specific antibody directed against the antigen responsible for its creation.

Viral Antigens and Antibodies

DR. A. W. DOWNIE The characterization of virus antigens is extremely complex and dependent in part on the interaction of virus antigen with antibody. The antigenic structures of vaccinia and influenza viruses have been the most extensively studied systems to date.

Vaccinia antigens

The vaccinia virus elaborates at least three specific antigenic substances, (1) a soluble antigen present in nature, with a molecular weight of 240,000, (2) nucleoprotein antigen, and (3) hemagglutinin. The presence of a neutralizing antibody observed in animals given the active virus has raised the question of the relationship of these known antigens to the immunizing property of the active virus particle.

The soluble antigen possesses both heat stable and heat labile components, producing two distinct antibodies that react with single antigen called the LS antigen.

The nucleoprotein antigen can be prepared by alkaline

traction of the virus, and probably constitutes at least half of the virus particle. The injection into rabbits of noninfective alkaline extracts of virus particles produces antibodies.

The hemagglutinin of vaccinia, antigenically distinct from LS antigen and nucleoprotein antigen, is probably lipoprotein in nature. It is associated with a particle some 6.5 μ in size.

While each of these antigens separately induces antibody formation in the rabbit, the antibodies do not neutralize the active virus nor confer immunity to the animals. Serum obtained from a rabbit immunized with active virus to which has been added the three separated antigens, thereby absorbing out specific antibodies, still possesses its protective or neutralizing antibody with titer unimpaired. The immunizing property of the active virus particle is not found in its component parts as known today and only infection or hyperimmunization with a live vaccinia virus will induce a high degree of immunity with the production of neutralizing antibody.

Influenza antigen

The influenza virus can be obtained in a relatively pure state because it is readily absorbed by the red blood cells of many species. However even the most highly purified preparations of influenza virus still contain antigens characteristic of the host from which the preparation is derived. Chick embryo isolates contain antigens characteristic of allantoic fluid, and mouse lung isolates contain antigens characteristic of normal mouse lung.

Antisera to the influenza virus will neutralize the virus, inhibit hemagglutination, and fix complement in the presence of the virus. Particles of smaller size than the virus have been prepared which have hemagglutinating properties.

Two antigens may be shown to fix complement. A soluble antigen is derived from infected tissue that is type-specific but not strain-specific, i.e., the soluble antigen of a type A strain will not fix complement with a type B antiserum, but will react with antisera to serologically different strains within type A. The second complement fixing antigen is associated with the virus particle and is strain-specific. Immunity to infection with influenza A viruses is largely strain-specific and the hemagglutinin-inhibiting activity of an antiserum closely parallels neutralizing activity.

Factors determining immunity

Immunity subsequent to recovery from virus infections is de-

pendent on the production and persistence of the specific antibody. Nonspecific factors may determine some degree of resistance and offer an explanation for the variation in resistance shown by a population of nonimmune individuals. Nonspecific virus-inhibiting properties of sera and tissue extracts seem to be associated with mucoproteins.

The presence and amount of specific antibody will not necessarily determine the degree of immunity to a virus infection under natural conditions of exposure.

The nature of the virus itself determines in some measure the immune response. Low immunity appears in some instances to be associated with a persistence of the virus in the tissues and an associated allergic reactivity to the inoculated virus. This situation gives rise to a chronic and sometimes relapsing type of infection.

Clinical influenza may occur in a person possessing antibody against the infecting strain. The influenza virus can invade the mucosa of the upper respiratory tract without gaining entry into the bloodstream. Immunity under these circumstances is dependent on the concentration of antibody at the point of entry (in the secretions of the respiratory mucosa). In generalized infections where viremia must occur serum antibody is more effective. The long duration of immunity following such virus diseases as yellow fever and the exanthemata has provoked much discussion. It has been suggested that the virus-virus antigen may persist in the tissue to provide a stimulus for further antibody formation. The poxviruses in birds, the lymphocytic choriomeningitis virus in mice, and the herpes simplex virus in man persist in tissues and remain latent until external circumstances upset the host-parasite relationship reveal the presence of the virus. As yet we have no epidemiologic, clinical, or experimental evidence to support the concept that the viruses of smallpox, yellow fever or childhood exanthemata persist in the tissues.

The Characterization and Production of Antibody Protein

DR. DAN H. CAMPBELL. The clinical investigator as well as the biochemist should have some working knowledge of the nature of antibody that is involved in the antigen-antibody reaction.

Size and shape of antibody

The molecular size of an antibody is an extremely important

consideration, for it has bearing on such phenomena as diffusion of antibody across the placental barrier as well as the blood-brain barrier and permits estimation of the number of molecules involved in skin reactions, hemolysis, or agglutination reactions. With the exception of an antibody found in the serum of horses sensitized with pneumococcus polysaccharide the molecular weight of antibody is about 160,000. Antibody is a cigar-shaped structure measuring 250 Å in length and 35 Å in diameter.

The electrophoretic properties of the antibody molecules that confer immunity and produce the usual serological reactions of flocculation and precipitation resemble those of gamma globulin. Antibodies not giving the usual serological reactions (e.g. reagin of allergic serum or the blocking antibody of anti Rh serum) migrate in an electrophoretic field with globulins of the α_2 or β variety.

Solubility and chemical composition

In experimental studies, the solubility of antibodies assumes importance. Generally the water-insoluble component increases during the immunization of rabbits and is reflected in a great amount of antibody in the water-insoluble portion of gamma globulin.

The chemical composition of antibody protein does not distinguish it from serum proteins nor permit differentiation between antibodies produced by differing antigens. Carbohydrate and lipid groups are integral parts of antibody molecules, with the lipid groups influencing only the rate of antigen-antibody interaction, and not the primary combinations that may occur. End-group analysis of terminal amino acids in rabbit antibody indicates a sequence of amino acids similar to that in normal gamma globulin—alanine, leucine, valine, aspartic and probably glutamic acid.

When an animal is injected with an antigen even as molecularly pure as crystalline ovalbumin the resulting antiserum contains antibody molecules differing widely in their immunological and physical properties. This heterogeneity of antibody is important in considering the reaction capacity and potentialities of serum, for the titer of an antiserum is the result of competing reactions by a heterogeneous population of antibody molecules.

Combining sites

Antibody-combining sites have been the subject of much study and speculation. Those antibody molecules involved in the final

tion of insoluble complexes are considered to have two combining sites. This finding has led to the concept that nonprecipitating antibodies therefore have only one combining site; however, no direct evidence for this is yet available. The relative positions of the combining sites are unknown. Removal of one half the diphtheria antitoxin molecule by pepsin digestion leaves all the original combining sites with the remaining half. It is conceivable that the number of sites may be the same in all antibody molecules but that the placement varies. If the sites are close together, only single antigen molecules can react, spatial considerations excluding other reactions. The antibody-combining site is relatively small, with an area of 500-700 Å²; hence metalloorganic complexes or ligands such as iodine may act as specific determinants. While the combining site has a highly specific complementary structure relative to antigenic determinants, the site does not influence the antigenic specificity of the molecules.

Factors influencing antibody formation

Speculation on antibody formation must acknowledge many factors. Some of the more important are (1) animal species—data obtained on rats are not entirely comparable to data obtained on rabbits or humans; (2) the chemical and physical nature of the antigen employed—in order to produce antibodies the material must be digestible *in vivo*; (3) the age and past history of the animal—the antibody-forming mechanism of young animals is limited and an occasional good response in test animal may reflect prior exposure to the antigen; (4) the type of substance containing trace of the component under study; (5) the type of test to be used to determine the presence and amount of antibody is important since some are more sensitive than others. If an excess of inhibiting antibody is present, high concentration of circulating antibody giving a precipitation or agglutination reaction may be missed.

When an animal is immunized, very little change occurs in the physical properties of the serum. With continuing immunization an increase in gamma globulin and sometimes beta globulin occurs. The electrophoretic patterns of three pools of rabbit serum are shown in figure 2. The highest titer serum has the greatest amount of gamma globulin with two distinct peaks. With strenuous immunization the concentration of gamma globulin, which normally constitutes 15 percent of the total serum protein, may rise to two or three times this value, with antibody constituting about half of the total gamma globulin. While the question of maximum anti-

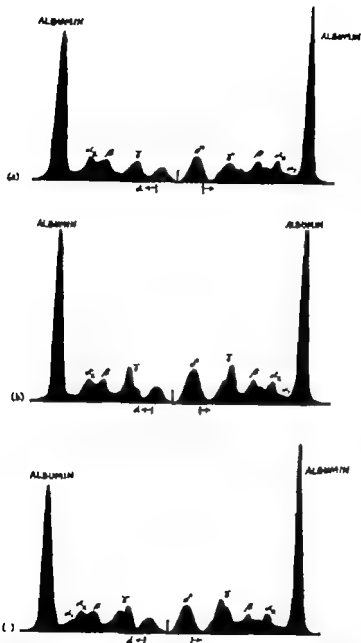


Figure 2 Electrophoretic patterns of serum from rabbits immunized against arsenic-azo-bovine globulin (RB₀) showing differences in the gamma globulin component. = 1 mg. of anti-R, b = 4 mg. of anti-R and = 5 mg. of anti-R per mL. of serum. Reprinted from The Journal of the American Chemical Society 73:4611 (1951)

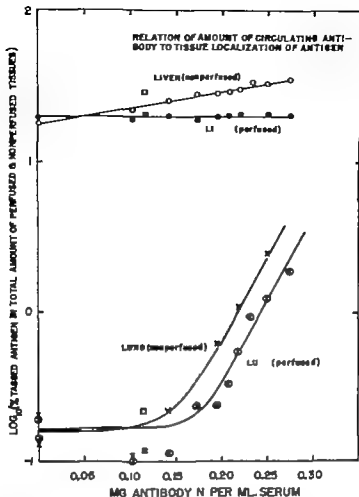


Figure 3. The relation of circulating precipitating antibody (anti-familia-ant-homocystin) to localization of homologous ^{59}Co labeled antigen. Reprinted from the Journal of Immunology 72 (51) (1954)

body forming capacity of the body has not been studied, it must be assumed, if there is maximum, that simultaneous immunization with multiple antigens will produce less antibody for given antigen than will an antigen injected alone.

The increase in gamma globulin after immunization cannot be entirely accounted for as antibody by serological testing hence it

is referred to as normal gamma globulin. Many immunologists, however consider all gamma globulin as antibody formed against some unknown antigen. When potent antigens are used, antibody formation can be very rapid. The injection of C-14 labeled leucine into rabbits immunized against ovalbumin results in the appearance of circulating radioactive antibodies in 24 to 48 hours. Once antibody is formed, the injection of additional specific antigen is followed by a rapid rate of antibody formation. The localization of antibody in tissues or organs is not influenced by circulating antibody until the concentration of antibody approaches 0.1 mg. per ml of serum (figure 3)

Theories of antibody formation

The two main theories of antibody formation remain in about the same state as they were ten years ago. One concept is that antigen directly shapes the gamma globulin molecule, either by simple mechanical molding or by changing the sequence of amino acids and thereby imposing specificity. The other concept regards antigen as influencing cell organizers, which in turn direct the synthesis of specific proteins. The first concept involves the continuing presence of antigen while the latter does not in antigen once the antigenic pattern has been imposed on cellular enzyme systems. Antigen retention in tissues thus assumes a very important role. Experimental study of this problem is difficult because antigen quickly disappears beyond chemical detection, but it may still be present and account for continuing antibody formation. If antigen persists in tissues it may do so in a partially degraded form, hence must be isolated and identified as such.

Metabolism of Antibody

DR. FRANK J. DIXON Since the rates of synthesis and degradation of antibody appear to correlate with the amount of antigen in the host, the study of antibody metabolism requires the use of an antigen which can be traced quantitatively *in vivo*. Purified heterologous serum protein antigens labeled with P^{32} would seem ideal for this purpose. They are readily traced, call forth a relatively simple immunologic response, equilibrate within the host's plasma protein pool, have access to all tissues, and are not appreciably concentrated or retained by the latter. Because they are catabolized rapidly they permit observations on antibody metabolism during

the presence of antigen, and later in its absence. Since these antigens do not persist long in the host, the synthesis of antibody is not complicated by prolonged antigenic stimulation, and the degradation of antibody is not influenced by continuous antibody-antigen union in the tissues. The antibody found in response to these antigens can be quantitated by the precipitin reaction.

Antibody anabolism

As shown in figure 4 the first indication of antibody production is an acceleration in the rate at which antigen is eliminated from the circulation. Following a primary intravenous injection into

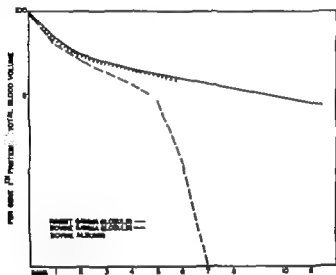


Figure 4. Disappearance of P^{32} labeled proteins from the blood of rabbits. Reprinted from the Journal of Allergy 24:552 (1953)

rabbits of P^{32} labeled rabbit or bovine gamma globulin or of P^{32} labeled bovine albumin, the homologous rabbit gamma globulin equilibrates with the intravascular and extravascular components of the plasma protein pool of the first 48 hours. It is subsequently lost from the blood at logarithmic rate with half life of five days, the rate at which homologous gamma globulin is catabolized. The blood level of bovine albumin is similar to that of rabbit globulin for the first seven day after

and then diminishes at a rate comparable to that observed for anti body decline. Whether or not this basal rate of synthesis continues in the absence of antigen cannot be established with tracer techniques. It seems unlikely that heterologous serum proteins so easily catabolized would persist in the host for months.

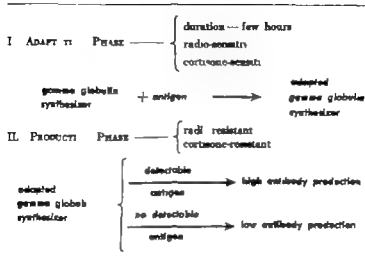


Figure 7 Phases of antibody production.

Phases of antibody synthesis

From these observations and from studies on the effects of x ray and cortisone on antibody production, the antibody response can be divided into two phases — an adaptation phase and a production phase (figure 7). The adaptation phase, of short duration, probably representing an initial period of adjustment of the host to antigen, is sensitive to cortisone and x ray. In rabbits the adaptation phase of antibody production can be inhibited by 400r whole body radiation (about one half an LD₅₀ dose of radiation). 5 mg of cortisone per kg of body weight per day. Radiation is most effective if administered prior to the antigen, and once this adaptive phase is complete, continued antibody response is relatively unaffected by radiation or cortisone. The adaptation phase probably represents some modification of the gamma globulin synthesizing mechanism under the influence of antigen, which results in the pro-

duction of altered globulin or antibody. The rate of production of antibody is in part related to the quantity of detectable antigen in the host.

The metabolic characteristics of the host determine the catabolic fate of proteins as illustrated in table 1. The half lives of gamma globulin and albumin are similar for a given species. Elevating the metabolic rate of rabbits by thyroid administration increases the rate of antibody catabolism and significantly decreases its half life.

Table 1

Half Life of I¹²⁵ labelled homologous gamma globulin and albumin

Species	Gamma Globulin			Albumin
	No. of subjects	Method of fractionation	Average half life with standard deviation—days	Average half life with standard deviation—days
Beef	4	Alcohol	21.2±1.7	20.7±1.1
Human				
6 mo.—8 yrs	11	Alcohol	20.3±4.1	
Adult	14	Alcohol	15.1±2.8	15.3±2.0
Dog	8	Alcohol	8.0±1.1	8.3±1.4
Monkey	10	Ammonium sulfate	6.6±1.6	
Rabbit	9	Alcohol	4.6±0.6	5.8±1.4
	9	Ammonium sulfate	5.7±1.2	
Guinea pig	13	Ammonium sulfate	5.4±0.9	
Mouse	27	Ammonium sulfate	1.9	1.2

Antibody catabolism

Antibody catabolism is dependent on at least one other factor—the presence or absence of specific antigen. In the absence of antigen, antibody is catabolized at the same rate as nonspecific gamma globulin. As determined by immunologic and isotopic tracer techniques, this rate varies considerably from one species to another (table 1). The slower metabolic rate, especially during the earlier months of life, may explain the slower rate of gamma globulin catabolism observed in infants and children, but it is likely that other unknown factors are also determinants in this situation.

When specific antigen is present in the host, circulating antibody and antigen combine forming complexes which are rapidly removed from the circulation and catabolized. When rabbits are

given passive transfers of I^{131} labeled rabbit antibody and then challenged with specific antigen, the labeled antibody is removed from the circulation within a few hours. Following its removal from the circulation the antibody is rapidly catabolized as indicated by the appearance of nonprotein-bound I^{131} in the urine. Similar studies of the fate of labeled protein antigens indicate that when antigen and antibody combine they are catabolized at comparable rates. This observation is not in keeping with the assumption that antigenic substances are not readily catabolized by the host and therefore persist and stimulate prolonged antibody synthesis.

The pattern of antibody catabolism in the presence of antigen depends in part on the physicochemical characteristics of the antigen and its distribution within the body. Antibody-antigen complexes formed and circulating within the intravascular and extravascular fluids are probably fixed and rapidly catabolized by circulating phagocytic cells. In contrast, rabbit antimouse kidney antibodies becoming fixed to the glomeruli of the mouse are lost at a half life rate of 20 to 21 days. Antibody so fixed is apparently protected from cells with great proteolytic activity.

Hormonal Influences on Antibody Mechanism

DR. THOMAS F. DOUGHERTY: The fact that there is so much conflicting evidence concerning the relationship of adrenal cortical hormones to the production of antibodies is due in part to a lack of delineation of the means of measuring the process of antibody production. At least three aspects of this process should be considered. Like other proteins, antibody must be synthesized in cells and, once formed, must be released to body fluids, eventually to be utilized, i. e. catabolized.

Effect on circulating antibody

It has been reported that administration of adrenal cortical hormones, stimulation of endogenous secretion of these hormones by administration of ACTH, may produce an increase of circulating antibody. In the first description of this effect, it was noted that the increase was of very brief duration and was variable in the extent to which it occurred. Since the presence of antibody had been

demonstrated in lymphocytes and since lymphocytes are destroyed following administration of or an increase in blood levels of adrenocortical hormones from endogenous sources, it was suggested that lymphocytolysis is accompanied by a release of antibody from this preformed source, although without implication that the adrenal cortex is essential to the synthesis of antibody. It was also pointed out that, although adrenal cortical hormones enhance the destruction of lymphocytes, antibody did not appear in adrenalectomized animals, and that therefore the mechanism of release of antibody was not dependent entirely upon the presence of the adrenal cortex. These early results have not been duplicated, although some investigators have confirmed the nonspecific anamnestic response induced by adrenal cortical secretions.

Evidence concerning the effect of the removal of the adrenal on the rate of production of antibody is conflicting. Several investigators have found that removal of the adrenals enhances antibody synthesis, others have reported that the formation, particularly of hemolysins, is definitely impaired in the adrenalectomized rat, and still others have reported that adrenalectomy is without effect upon the antibody-forming mechanism. The administration of adrenal extracts for a prolonged period of time has been reported to increase the level of antibody while ACTH and cortisone, given for any prolonged period of time, have been demonstrated to depress antibody formation. In man, particularly administration of these hormones is without any effect on antibody titer.

In a study done by Blum, the formation of products of antibody to pneumococcal polysaccharides after administration of ACTH (17 cases) or of cortisone (12 cases) was not depressed, but actually slightly increased compared to three control subjects. Little effect of these hormones on production of agglutinins to typhoid bacilli was found, although it should be noted that isohemagglutinins were increased four-fold in 4 of 11 hormone-treated patients. Serum globulins decreased in several of the hormone-treated patients in spite of the fact that they had an increase in antibody.

It is apparent that there can be no point of general agreement at the present time concerning the mechanism of adrenal cortical hormones in either the synthesis or the release of antibody. However it has been demonstrated that antibody is formed and released in the absence of the adrenal cortex. Under proper conditions of experiment, antibody synthesis can be markedly depressed by the administration of very large amounts of adrenal cortical hormones. Cortisone and hydrocortisone have been shown

to diminish markedly the capacity of immunized animals to synthesize antibody. This effect has not as yet been confirmed in humans, and it is believed that the dosages required to produce a suppression of antibody synthesis are far greater than those used either therapeutically or experimentally in human patients. It seems that a high level of circulating adrenal hormones must be attained prior to the administration of the antigen in order to suppress antibody formation. If adrenal hormones are given even shortly after antigen is administered, relatively little suppression is brought about.

The author has analyzed this from the standpoint of dose-response phenomenon. Single graded doses of antigen were administered to inbred mice on a weight basis, some of the immunized animals received daily graded doses of either ACTH or cortisone during the entire period of study. Instead of the usual serial dilution method of establishing response, a technique was used whereby the extent of antibody suppression was graded according to a quantal response, that is, based upon a plus or minus appearance of antibody at a single dilution. The degree of suppression of antibody was determined for a very large number of animals at intervals of 4, 9, 12, and 17 days following administration of single dose of antigen.

These experiments revealed that the degree of antibody suppression is directly dependent on the relation of the amounts of antigen and hormone administered, i. e., the greater the immunizing dose, the greater the amount of hormone required to suppress antibody. Antibody is suppressed most effectively after four days, and thereafter begins to appear despite continued hormone treatment. Complete suppression of antibody in the early period requires dosages greater than those which are, weight for weight, used therapeutically in human disease. In spite of almost complete inhibition of antibody in the circulation, the incidence of anaphylactic shock is only moderately reduced, indicating that antibody is present in tissue when it cannot be demonstrated in blood. This evidence supports the suggestion of Mirick that "the beneficial effects of ACTH and cortisone in treating hypersensitivity and related diseases in man are not to be explained by suppressed antibody productions".

Animals producing antibody to ovalbumin were administered, at the point of maximum concentration, enough ovalbumin to reduce the titratable antibody to zero. Subsequent cortisone administration resulted in the rapid reappearance of antibody in the

circulation, whereas the noncortisone-treated controls did not manifest circulating antibody until 24 to 48 hours later. The secondary response in the control animals, however, produced higher titers than those of the cortisone-treated animals. This evidence seems to indicate that cortisone can bring about release of preformed antibody and can produce a decrease in the rate of antibody synthesis, as shown by the diminished amount appearing in the blood during the secondary response.

Effect at cellular level

Some investigators have suggested that reticuloendothelial cells are antibody producers; others have supported the possibility that plasma cells and/or lymphocytes may be the main site of synthesis of these proteins. It is possible that all three of these cell types may play some role in the overall synthesis of antibody protein. In any case, the mechanism by which adrenocortical hormones may suppress antibody synthesis is intimately related to the effect of these hormones on the cells which produce antibody.

Reticuloendothelial cells, which incorporate antigen, undergo differentiation to various types of cells such as the lymphocyte and the plasma cell. The offspring, such as the stages of differentiation of lymphocytes and possibly plasma cells, actually carry out most of the synthesis of antibody protein. This process is comparable to that of the synthesis of hemoglobin which begins in proerythroblasts and continues throughout the various stages of differentiation of erythroblastic cells. Thus, the direction of the type of antibody protein synthesized is predetermined in the reticular cell which phagocytized the antigen.

It is well known that there is tremendous inhibition of lymphocytopenia resulting from excess adrenocortical hormone secretion or administration of the hormones of the C-11 oxy or hydroxy-type. The essence of the problem would seem to be that the three types of agents capable of suppressing antibody formation may act similarly. For example, the well-known suppressive effect of x-radiation on lymphocytopenia is accompanied by inhibition of antibody synthesis. Complete starvation induces an inhibition of lymphocytopenia which, oddly enough, is mediated by way of the adrenal cortex suppression of antibody synthesis also accompanies this type of suppression of lymphocyte formation. Other agents that inhibit lymphocytopenia, even in the absence of the adrenal cortex, as do the nitrogen mustards, also inhibit anti-

body synthesis in the absence of the adrenal cortex. Pyridoxine deficiency even in the absence of the adrenal cortex, brings about the inhibition of lymphocytopoiesis and an accompanying inhibition of antibody synthesis. In each of these inhibitory responses it appears that if antigen is given before the suppression of lymphocytopoiesis there is less inhibition of antibody synthesis.

Several of these agents, e. g. ACTH and x radiation, on the other hand, do not significantly diminish plasma cell formation. Many reports have verified the finding that ACTH actually enhances plasma cell formation. Plasma cells are not decreased by large doses of ACTH to the same extent as lymphocytes. For these reasons, in part, it would seem that much of the suppressive effect of the adrenal hormones on antibody synthesis is related to and is a consequence of the suppression of the formation of lymphocytes. It should be noted that there appears to be no question but that plasma cells as well as lymphocytes contain antibody. However, the cellular content of this protein cannot be taken directly as an indication of the role of the particular cell in the synthesis of this protein. It should also be noted that several authors have now cultivated lymphocytes in tissue culture and have demonstrated that these cells will synthesize antibody.

There is some evidence that the adrenocortical steroids bring about release of preformed antibody. The role of adrenocortical steroid in bringing about the catabolism of the antibody when it is delivered to the circulation is not well understood. It is apparent, however, that they do not greatly enhance the removal of this protein from the circulation. On the other hand, the evidence seems to be very clear-cut that the adrenal hormones, like other antilymphocytopoietic and environmental conditions, bring about a suppression of synthesis of antibody protein and inhibition of lymphocytic differentiation.

Discussion

DR. ENRICH. This session on basic concepts of immunity and hypersensitivity is indicative of our slow rate of progress in understanding and clarifying this extremely complex and confusing subject. Terminology and definitions must be precise so that confusion is not compounded.

DR. CAMPBELL. Two important areas of need for more understanding are (1) the nature of noncomplexing antibodies and their contribution to the early stages of immunization, and (2) the per-

sistence of antigen. Many experiments directed at the latter problem have resulted in efforts to locate the original antigen. Since all antigenic materials are digestible, fragmentation must occur. The task of localizing these fragments of necessity must be done within the body since degradation of antigen does not proceed in the same manner in *in vitro* experiments.

DR. DIXON: As yet, we do not have adequate antigen tracer techniques to finally answer this question. On the one hand, antigenic fragments are often difficult to trace and on the other hand, we may detect foreign materials by our tracer techniques which are retained in tissues but are not stimulating antibody production. We are a long way from unraveling this problem.

DR. DOUGHERTY: In discussing the persistence or retention of antigen I should like to cite the findings from an experiment with lymphosarcoma cells in mice. A lymphosarcoma tumor transplanted into a mouse previously immunized with tumor cells can be transferred to other mice for a total of three passages.

DR. ROBERT A. GOOD: It is our view based on correlative morphologic-immunologic studies that there is some truth in each of the current cellular theories of antibody production. In the face of current evidence, some of it direct, there can be no doubt that plasma cells produce or release antibody. When cells of the body begin to produce antibody or gamma globulin, this physiologic function gains morphologic expression. Evidence obtained from morphologic studies indicates that in response to antigenic stimulation plasma cells can be derived from undifferentiated reticulum cells, periadventitial mesenchymal cells, lymphocytes, monocytes and even microglia. Under ordinary experience with an initial injection of antigen plasma cells seem to derive largely from the multipotent reticulum cells of the lymph node, spleen and bone marrow. Under more extreme stimulation as during the course of the secondary response particularly in the area of the local inflammatory process induced by exposure to specific antigen to which an animal has previously been immunized, these cells may derive from lymphocytes, monocytes, microglia or periadventitial mesenchymal cells. As these various cells of mesenchymal origin begin to form antibody their cytoplasm accumulates ribonucleic acid, hence its basophilia. The nucleus becomes eccentric, probably as a function of the secretory activity. A clear area or hole develops in the perinuclear region as the mitochondria, Golgi apparatus and cytocentrum displace the ribonucleoprotein of the cytoplasm, and the nucleus becomes that of a cell engaged in vigorous metabolic

activity. These morphological features are the criteria by which the pathologist defines plasma cells. It seems from our data then that many cell types, including perhaps even reticuloendothelial cells, may form antibody but that when they are actively engaged in this function they take on the morphological appearance of plasma cells. With this view the plasma cell, derived from any source, becomes the morphological sign of antibody production.

Studies on the Mechanisms Involved in the Shwartzman and Arthus Reactions

The Shwartzman reaction

Dr. Good: Future research will doubtless bring understanding of the destructive processes associated with infection, allergy and hypersensitivity in precise physiologic and biochemical terms. Progress toward definition of tissue damage in this way requires controllable models of necrotizing processes which can be manipulated with facility and the manipulation of which promises to shed light on disease mechanisms in man. Such a model is the Shwartzman reaction. This is a two-stage reaction resulting in hemorrhagic necrosis of the skin. This lesion is produced whenever an intradermal injection of a gram-negative bacterial endotoxin is followed after a suitable time interval by an intravenous injection of the same or similar toxin.

A mild inflammatory response characterized by erythema, edema, and slight induration, which develops around the site of the initial intradermal injection of toxin, is called preparation. This sequence of events following the intravenous injection of endotoxin is called provocation. A minimum of eight hours is required for skin preparation, and the hemorrhagic necrosis occurs three to four hours after the intravenous injection of endotoxin. The Shwartzman reaction is immunologically nonspecific; preparation and provocation can be carried out with filtrates of unrelated microorganisms.

Characteristics of prepared skin

Morphologically the prepared skin at the site of intradermal injection of endotoxin has the appearance of inflamed skin. Infiltration with polymorphonuclear leucocytes is noted with conspicuous perivascular cuffing. Some mononuclear cells may be observed. Blood vessels for the most part are patent. In some capillaries and venules, margination and diapedesis of leucocytes may be seen. There are no morphological findings that differentiate preparation for the Shwartzman reaction from an ordinary in-

flammatory response. However prepared skin does not stain in response to an intravenous injection of trypan blue, in contrast to normal skin which turns blue, or to an area of inflammation which shows enhanced permeability to vital dyes.

Metabolic abnormalities can be demonstrated in prepared skin. Intense aerobic glycolysis with consequent accumulation of lactic acid and local lowering of pH have been described. Substances such as foreign proteins giving rise to an inflammatory reaction, but incapable of preparing the skin, do not enhance lactic acid production to the same degree as do gram-negative bacterial endotoxins.

Inhibition of the reaction

Inhibition of the Schwartzman reaction in rabbits by pretreatment with nitrogen mustard, x-ray or benzene (agents known to produce leucopenia) demonstrates the essential role of poly-

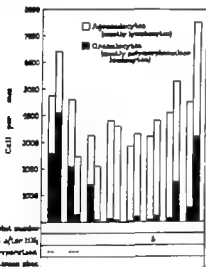


Figure 8. Relation of inhibition of Schwartzman reaction to fall in granulocytes and administration of nitrogen mustard. Reprinted from the Journal of Experimental Medicine 93:49 (1951)

morphonuclear leucocytes in this process. Nitrogen mustard in a dose of 1.5 mg. per kg. of body weight produces a granulopenia maximal in 72 to 96 hours. The Schwartzman reaction is regularly inhibited during the period of maximal leucopenia but may occur both prior to the fall in granulocytes and following their reappearance in the circulation (figure 8)

That inhibition of the Schwartzman phenomenon is not due to some other toxic effect of nitrogen mustard can be demonstrated by

protection of the femoral marrow from its action. A clamp placed on the abdominal aorta of the rabbit for a 10 minute period during and after the injection of the mustard all was fixated of the nitrogen mustard by those areas of the body above the clamp, sparing the femoral marrow below. While these animals show evidence of toxicity to nitrogen mustard they do not develop granulopenia. The prepared skin sites give characteristic Shwartzman reaction (table 2).

Blocking the granulopenia induced by benzene by simultaneous treatment with sulfapyridine allows these rabbits to exhibit a Shwartzman reaction. Presumably because of the neutropenia produced, the Shwartzman reaction does not occur in animals treated with benzene alone.

The increased glycolysis and lactic acid production of skin prepared for the Shwartzman reaction are diminished in rabbits made leucopenic by pretreatment with nitrogen mustard. Prepared skin, impermeable to trypan blue in the classical Shwartzman reaction, stains with this vital dye in animals having polymorphonuclear leucopenia. Thus the polymorphonuclear leucocyte seems to be essential for most of the changes described as characteristic of prepared skin.

Leucocyte changes

Polymorphonuclear leucocytes may also play a vital role in the development of the hemorrhagic necrosis that follows the provoking injection of toxin. Immediately after an intravenous injection of toxin, thrombocytopenia and granulopenia are observed. This phenomenon is attributed to the development of adhesiveness of leucocytes and platelets, with removal of these fixed elements from the circulation as clumps or plugs in the capillary bed of the lungs and other organs. As Stetson has shown, the capillaries and venules in the prepared skin site become occluded by leucocyte-platelet thrombi, and the necrosis of the vessel wall as subsequent hemorrhagic necrosis seems to be a function of this event.

Effect of anticoagulants

The concept that thrombus formation is essential as a stage in the development of the Shwartzman reaction is supported by study of the effect of heparin on the reaction. Intravenously administered aqueous heparin, or subcutaneously injected depot heparin in quantities that produce incoagulability of rabbit blood for four hours,

Table 2

Susceptibility to the Shwartzman phenomenon, following treatment with HN₂ of rabbits in which the femoral marrow had been protected against the HN

	Rabbit No.	Total leucocytes		Polymorphonuclear leucocytes		Intensity of Shwartzman phenomenon
		Before all preparation	Before intravenous challenge	Before 1/2 preparation	Before intravenous challenge	
Protected ^a rabbits	1	cells/cm ³ : 6600	cells/cm ³ : 8550	cells/cm ³ : 5300	cells/cm ³ : 2140	++
	2	6850	4550	5150	2000	++
	3	5500	3550	4850	2130	++
	4	4900	6400	2600	3200	++
Sham-injected rabbits	5	4350	3650	340	73	—
	6	2110	2550	21	51	—
	7	3600	3750	410	211	—

+++, ++, +, and 0: development of typical hemorrhagic necrosis at sites following the intravenous challenge — indicates absence of any hemorrhagic necrosis following the intravenous challenge

completely blocks the development of the Schwartzman reaction when given at the time of provocation (table 3)

Administration of heparin during the period of preparation is without effect. In the doses used it did not affect the leucocyte or platelet count nor prevent the polymorphonuclear leucopenia that follows intravenous injection of toxin. While heparin had profound influence on the Schwartzman reaction it did not inhibit the lethal effect of a single large dose of intravenously injected toxin.

Table 3

Prevention of local Schwartzman reaction with heparin

Group	Subsides injected	Positive reaction
No heparin	30	29
Heparin I.V.	18	2

Other anticoagulants such as Tromexan® and dicumarol are also effective in blocking the Schwartzman reaction. These observations support the belief that vascular occlusion constitutes part of the mechanism involved in the development of hemorrhagic necrosis of this type.

Vascular spasm

Vascular spasm may also be a factor in the development of the Schwartzman reaction. The injection of adrenaline, 1 ml of 1:10,000 dilution, into the skin and subcutaneous tissue surrounding a prepared skin site results in a lesion virtually identical to the Schwartzman reaction. Hemorrhagic necrosis does not occur if the injection of adrenaline is made intracutaneously subcutaneously in an area far removed from the site of preparation.

The Arthus reaction

The Arthus reaction, like the Schwartzman reaction, is a skin lesion characterized by hemorrhagic necrosis. Although produced by entirely different methods the observations of Stetson indicate that this phenomenon also involves the following mechanisms:

1. Perivascular accumulation of polymorphonuclear leucocytes in the injected skin area.

2. Aerobic glycolysis at the site of antigen injection.
3. Adhesiveness of leucocytes and platelets from contact with the provoking agent.
4. Leucopenia following the injection of the provoking agent + antigen.
5. Leucocyte-platelet thromb in the capillaries and venules of the injected area.
6. Hemorrhage from vessels occluded by cellular thrombi.

Rabbits previously sensitized to ovalbumin and prepared for the Schwartzman reaction by the intradermal injection of meningococcal toxin show hemorrhagic necrosis 24 hours after an intradermal injection of ovalbumin, at the site of the initial ovalbumin injection and at the site of injection of meningococcal toxin.

Effects of cortisone and ACTH

Contrary to other findings reported, cortisone given in any dose range to rabbits from our stock colony does not inhibit the Schwartzman phenomenon. While ACTH does result in slight alter

Table 4

Effect of cortisone and ACTH on the Schwartzman reaction

Group	Dose mg./kg. body weight	No. rabbits	No. positive
Cortisone (daily for 4 days)	25	10	9
	20	8	8
	10	8	4
	8	8	6
	5	6	5
	2	6	8
	0	16	15
ACTH (every 11 hours for 4 days)	10	8	8
	8	6	6
	5	5	5
	2	6	6
(every 8 hours for 3 days)	40	8	5
	0	22	1

ation in the gross features of the reaction it does not prevent the local or generalized Shwartzman reaction (table 4). Instead, in cortisone-treated rabbits a primary hemorrhagic necrosis occurs following an intradermal injection of toxin without a second intravenous injection of endotoxin.

The primary hemorrhagic reaction to toxin in cortisone-treated rabbits is prevented by pretreatment of rabbits with nitrogen mustard

by treatment with heparin. In its morphologic features this skin lesion seems to evolve through the same mechanisms that are involved in both phases of the Shwartzman reaction. Following this observation it was observed that a single intravenous injection of toxin in cortisone-treated rabbits results in the development of hemorrhagic necrosis in the kidneys. Normal rabbits rarely manifest this lesion after a single injection of the toxic material. Bilateral cortical necrosis of the kidney is characteristic of the generalized Shwartzman reaction, and was the lesion regularly produced by a single injection of toxin in cortisone-treated rabbits. This condition was not so regularly observed in ACTH-treated rabbits following a single injection of toxin. Whereas cortisone interferes with the shock producing and lethal action of gram-negative bacterial endotoxins, both cortisone and ACTH enhance susceptibility of rabbits to the necrotizing action of toxin.

Effect of thorotrast

Some rabbits appear to be more resistant to the damaging effects of endotoxins than others, and the repeated injection of small doses results in the development of a high degree of resistance to the Shwartzman phenomenon. Reticuloendothelial blockade produced by the intravenous injection of thorotrast abolishes both the naturally occurring state of resistance and acquired resistance.

Susceptibility of normal rabbits to the lethal effects of toxin can be increased more than 1000-fold by prior injection of thorotrast. These observations are interpreted as evidence that the reticuloendothelial system plays an important role in the body's defense against toxins of this type.

Implication of the Generalized Shwartzman Reaction

DR. LEWIS THOMAS The generalized Shwartzman reaction occurs in the rabbit when two intravenous injections of gram-

negative bacterial endotoxin are spaced 18 to 24 hours apart. As in the local Shwartzman reaction, it is essential that there be two injections, and that adequate time be allowed between the injections. The reaction is not due to the cumulative effect of the two injections, since it fails to occur with a single dose of much larger quantities of toxin than is effective in two divided doses. A characterizing manifestation is bilateral cortical necrosis of the kidneys, a lesion that appears to be initiated, within four hours after the second injection of toxin, by occlusion of the glomerular capillaries with masses of homogeneous eosinophilic material resembling fibrinoid.

The presence of circulating polymorphonuclear leucocytes appears to be necessary for the reaction. In rabbits with leucopenia caused by nitrogen mustard, bilateral cortical necrosis of the kidneys cannot be produced. When leucopenia is prevented by shielding the femoral marrow from the action of nitrogen mustard, renal necrosis occurs as in untreated rabbits. The role of polymorphonuclear leucocytes in the reaction cannot be explained.

Heparin prevents the occurrence of the generalized Shwartzman reaction, apparently by preventing the deposition of occluding material within the glomerular capillary lumen.

Cortisone does not protect animals against the reaction. On the contrary cortisone treatment causes the animals to react to a single injection of endotoxin by developing renal cortical necrosis. This observation suggests that cortisone may interfere with a normal protective mechanism for the detoxification of such substances. In view of the known morphologic effects of cortisone on tissues of the reticuloendothelial system, the possibility that the altered response to toxin might be mediated by an effect on this system was investigated.

An intravenous injection of colloidal materials known to be taken up by reticuloendothelial cells, including thorotrast, trypan blue, colloidal iron saccharate, and colloidal carbon, was followed by a single intravenous injection of endotoxin. The outcome was similar to that in cortisone-treated rabbits, one dose of toxin resulting in bilateral cortical necrosis of the kidneys in a high proportion of the animals. Reversal of the order of injection, with the colloidal substances given a few hours after toxin instead of before, produced no kidney lesions.

Limitation of protective function of reticuloendothelial system

On the basis of these and other observations the following

hypothesis was constructed to account for the necessity for two intravenous injections of endotoxin in the generalized Shwartzman reaction. The animal responds to the first dose of toxin with a protective mechanism that involves the functioning of cells of the reticuloendothelial system. As the result of saturation of capacity this system becomes temporarily unable to repeat its function, and the second dose of endotoxin is free to act directly on other tissues such as the kidney which are not affected in the normal animal. A similar state of vulnerability is caused by cortisone, thorotrast, trypan blue, and colloidal iron and carbon. In animals treated with these materials the protective function of the reticuloendothelial system is temporarily impaired, and a single dose of toxin causes renal necrosis.

The occurrence of a similar situation in animals with systemic infection by group A hemolytic streptococci has been investigated. When streptococcal infection is followed by a single injection of endotoxin severe generalized Shwartzman reactions are produced, accompanied by fibrinoid necrosis of the coronary arteries, which resembles the arterial lesions commonly ascribed to "hypersensitivity."

A Pattern of Tissue Reaction in Hypersensitivity

DR. CAROLYN F. PIEL. Increased knowledge of the role of hypersensitivity in human disease has greatly stimulated endeavor to produce experimental models of such diseases. These models would then not only be available for investigation of therapeutic agents, but permit detailed study of the pathogenesis of clinically important entities. Outstanding in this respect are the innumerable observations involving attempts to produce renal disease in laboratory animals.

Of the variety of methods employed in the production of experimental nephritis, bacterial injections alone or bacteria mixed with homologous kidney emulsions have been widely utilized. The results have been singularly unrewarding and difficult to reproduce.

Heterologous serum in experimental renal disease

Another method that has proven more profitable involves the use of heterologous proteins. Intravenous injection of whole horse

or bovine serum or the purified fractions, globulin and albumin, produces a renal disease that many observers have compared with human glomerulonephritis. The experimental disease is characterized by a latency of 7 to 10 days between the time of injection and the onset of symptoms including proteinuria and pathologic findings such as proliferative changes of the glomerular tuft. In addition, animals injected with heterologous protein have lesions elsewhere in the body periarteritis, myocarditis and valvulitis.

By applying special histochemical techniques to kidneys removed from rabbits injected with whole horse serum, the proliferative change is noted to involve the mesenchymal tissue of the glomerulus, which lies between the capillary loops, rather than endothelial cells. This tissue reaction is not typical of human glomerulonephritis but rather of a generalized mesenchymal reaction such as is seen in serum sickness.

The renal lesions produced by heterologous protein depends on the ability of the antigen to reach the kidney, the ability of the recipient animal to produce specific antibody, and the occurrence of antigen-antibody reaction in the glomeruli. Purified albumin produces less renal disease than globulin, probably because its smaller molecular size allows considerable removal from the bloodstream prior to reaching the kidney. Hyaluronidase likewise depresses the renal lesions by facilitating the removal of antigen from the blood stream. Cortisone, x radiation, nitrogen mustard, colchicine and low protein diet all inhibit the occurrence of nephritis, probably on the basis of their antiphlogistic effect and their interference in production of antibody.

Use of nephrotoxic serum

Nephrotoxic serum prepared by repeated injections of emulsified kidney into animals of another species produces renal disease on injection into animals of the donor species. Nephrotoxic serum is highly species- and organ-specific. The antigenic portion of the kidney resides in the membranous section of the glomerulus. Adsorption of such sera by kidney emulsion *in vitro* produces a loss in nephrotoxicity. Localization of the site of antigenicity was made possible through observations demonstrating that cortical emulsions produce renal disease more readily than medullary emulsions. By differential centrifugation the glomerular fraction was found to be 20 times more antigenic than a tubular fraction. Further subdivision of the glomerular fraction localized the antigenic site to the membranous portion. Kidney emulsion incubated with trypsin results

in a clear stable solution, nonantigenic but capable of inactivating nephrotoxic serum. It is postulated that the antigen is protein-bound polysaccharide or lipid.

Further confirmation of the site of antigen localization has been obtained from tagged antibody studies. Mice injected with radioiodinated antikidney globulin show localization of radioactivity in the glomeruli. Fluorescein-tagged globulin from serum prepared using whole kidney produces fluorescence of the cytoplasm of the epithelium of the glomerular capillaries and cortical tubules. Globulin prepared from serum following glomerular tissue injection and tagged with fluorescein results in fluorescence of glomerular structures only.

Nephrotoxic fowl serum

Nephrotoxic serum prepared in fowl duck produces in dogs, rabbits, guinea pigs and rats a disease with two distinctive features, (1) a latency period of one week before onset of symptoms, and (2) hematuria. Rabbits injected with nephrotoxic duck serum may fix the duck serum in their kidneys. Five to seven days later the formation of rabbit and duck serum antibodies, may interact with the duck serum fixed in the rabbit kidney and result in nephritis. Oliguria, proteinuria, cylindruria and moderate edema characterize the clinical course of this disease. Some animals develop hypertension, urea retention, and decrease in glomerular filtration rate.

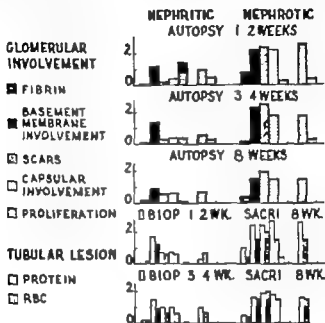
Nephrotoxic rabbit serum

Renal disease produced in rats following the injection of nephrotoxic rabbit serum differs from that produced by nephrotoxic duck serum in immediate onset of disease and lack of hematuria. By varying the potency of the rabbit serum two types of disease response can be obtained. One has proliferative changes comparable to human glomerulonephritis, occurring after latency of one week; the other occurring immediately after serum injection, has basement membrane involvement comparable to nephrosis.

The results of a study designed to establish whether or not these two types of tissue response remain distinct entities or merge into one another are summarized in figure 9. The changes studied are graded one to four plus, and the influence of biopsy procedures on the rate of progression are indicated. In general, the histologic changes are less severe in the nephritic group. In the early weeks of study the nephritic group shows more proliferation and red blood

cells in the tubular lumina. Basement membrane involvement in the nephrotic group tends to subside by the eighth week. Biopsy at one to two weeks intensifies the basement membrane changes. Biopsy at three to four weeks has less influence on outcome.

The influence of diet, stress, enzymes, and cytotoxic agents on the course of this disease in rats has been difficult to evaluate. Recovery



Figur 9 Average histologic and urinary changes in the kidneys of rats given two levels of nephrotoxic rabbit serum.

If rats given low protein diet has been recorded. Nitrogen mustard has been reported to depress the proteinuria of nephrotoxic serum disease. Cortisone and ACTH administration prior to or following the injection of nephrotoxic serum depresses edema, proteinuria, degenerative and proliferative changes. Cortisone in doses greater than 2.5 mg. per 100 gm. of body weight aggravates the disease.

Discussion

Dr. Goon: Is the formation of fibrinoid material in the glomeruli of animals given nephrotoxic serum inhibited by heparin administration?

DR. PIEL: Studies of nephrotoxic disease by Dr. Ehrlich indicate that heparin will prevent the production of thrombi.

DR. GOOD: Does such inhibition alter the severity of the renal lesion? Some of the thickening of the basement membrane may arise on this basis.

DR. PIEL: Inhibition of fibrinoid formation should alter the severity of the renal lesion. Animals that have been bled reflect a more severe disease probably due to the nonspecific production of fibrin thrombi.

DR. GOOD: We vacillate between calling this material fibrin and fibrinoid. Rarely on histochemical study does it have a fiber structure. Perhaps it is in a stage of fibrin development.

DR. EHRLICH: We don't know the difference between fibrin and fibrinoid. Relative to the use of heparin we have noted that heparin eliminates gross edema completely.

DR. GERMUTH: Does not the very fact that heparin inhibits the deposition of material suggest that it is fibrin? When blood capillaries undergo thrombosis, fibrin is often noted.

DR. EHRLICH: In experimental nephrosis as seen in the rat we do not necessarily have fibrin. The material accumulating on the basement membrane seems to be serum protein.

Influence of Cortisone on the Pathogenesis of Anaphylactic Hypersensitivity

DR. FREDERICK C. GERMUTH, JR.: Allergic phenomena like the immune processes of acquired resistance are mediated through antibodies. Allergy appears when the interaction of antigen and antibody produces injury to cells of the animal. Immunity occurs when this interaction alters microbial cells. Allergy and immunity are therefore different biologic expressions of the same immunologic phenomenon.

Allergy and fixed antibody

Allergy may manifest itself in a variety of ways. In some forms of allergy such as acquired hemolytic anemia and idiopathic thrombocytopenic purpura, antibodies are directed against the surfaces

of specific tissue cells which have been modified by the offending antigen. In drug allergy of the contact dermatitis type and in the allergy that follows infection, it would appear that antibody is carried in or on lymphocytes. Antibodies in allergy may be more or less firmly attached to cells, and free antibody in the serum may be slight or absent. In all of these instances, the result is damage to a specific cell of the body.

Allergy and circulating antibody

Exposure of the animal body to foreign protein antigen evokes the formation of circulating plasma antibodies which are not directed against any particular cellular component of the body. This type of allergic state induced by the formation of circulating antibodies is known as anaphylaxis. Here the interaction of circulating antibodies with antigen in the tissues leads to contraction of smooth muscle and damage to the cardiovascular system. These alterations are familiar in the form of asthma and allergic arteritis in man, and in the form of anaphylactic shock, the Arthus reaction and serum sickness in experimental animals.

Anaphylactic shock results from the intravenous injection of antigen into a sensitized animal. extremely small amounts of antibody are required to produce shock.

Intracutaneous injection of antigen into a sensitized animal is usually followed by local inflammatory reaction characterized by redness and edema and, in its more severe form, hemorrhage and necrosis. This symptom complex is known as the Arthus reaction.

Large quantities of antigen such as foreign serum injected intravenously into a normal animal may be followed, after an incubation period of several days, by the onset of fever and the appearance of severe damage to the cardiovascular and renal tissues. This state of protracted anaphylaxis is known as serum sickness.

Effects of hormones

The administration of daily doses of cortisone, ACTH or cholesterol (5 mg. per kg. of body weight) to rabbits injected daily intracutaneously with 1 mg. of crystalline egg albumin produced marked suppression in the Arthus reaction in those rabbits receiving cortisone. Serum antibody concentrations were reduced by the administration of ACTH while cortisone produced almost complete inhibition of antibody response. Failure of ACTH to alter the Arthus reaction seems dependent upon its inability to suppress anti-

body below a level of 40 micrograms of antibody nitrogen per ml. of serum.

Since cortisone exhibits anti-inflammatory action, its inhibiting effect on the allergic state may be related to an altered skin reactivity. Rabbits treated with cortisone and tested for passive Arthus reaction from antibody administered directly into the skin or intravenously show no effect of cortisone on the Arthus reaction (figure 10). These findings led to the conclusion that the inhibition of the active Arthus reaction by cortisone is effected by hormonal suppression of circulating antibody. Since circulatory antibody concentration can be reduced by either decrease in antibody synthesis or an increase in

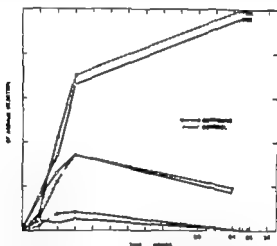


Figure 10. Effect of cortisone on passive Arthus reaction. Rabbits given subcutaneous albumin rabbit serum intravenously and skin-tested with (1) 0.01, (2) 0.1, and (3) 1.0 mg. of bovine albumin at one-half hour intervals.

the rate of antibody destruction, the mechanism by which cortisone administration alters antibody level requires further study. As shown in figure 11, cortisone is without effect on the rate of disappearance of antibody from the serum of passively sensitized rabbits, suggesting that cortisone suppresses antibody formation.

The mechanism by which cortisone suppresses antibody formation is obscure. Decreased antibody formation may have some relation to the extensive atrophy of the lymphoid tissue found in ACTH and cortisone-treated animals.

Large doses of cortisone are without effect on either active or passive anaphylactic shock in the guinea pig. Since the quantity of antibody required for shock is small, inhibition of antibody synthesis by cortisone does not inhibit shock.

Serum sickness

Advances in the understanding of the relationship between

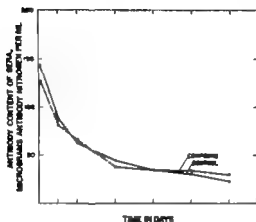


Figure 11 The influence of cortisone on the rate of antibody disappearance from the serum of passively immunized rabbits.

Table 5

Experimental lesions induced in rabbits 12 days after intravenous bovine albumin

Lesion	Percent
Acute glomerulitis	80
Focal accumulation of leucocytes in intima of pulmonary arteries or ascending aorta	72
Endocarditis (aortic and mitral valves)	54
Necrotizing arteritis (predominantly coronary)	28

tissue lesions and immunologic changes associated with serum sickness are possible when use is made of chemically homogeneous antigens instead of mixtures. Rabbits given single intravenous injection of crystalline bovine albumin (0.25 gm. per kg. of body

weight) show a high incidence of cardiovascular and renal lesions (table 5). Granulomatous lesions are observed in the spleen and lymph nodes of more than one half of the experimental animals.

The relationship among the time of onset of tissue lesions, the clearance of antigen from the serum, and the development of free antibody is shown in figure 12. Bovine albumin is eliminated in three phases and is followed by the appearance of antibody. Tissue

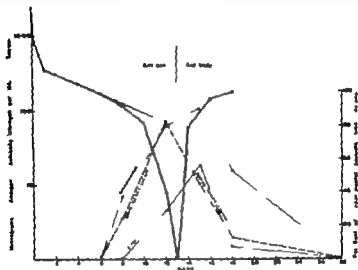


Figure 12. Relationship between the disappearance of antigen and appearance of antibody to the onset of tissue lesions in experimental serum sickness.

lesions are first observed during the third or immune phase of antigen elimination, when antibody formation is proceeding in the tissues. About the time that the immune phase is complete, tissue lesions are most severe and of highest incidence. With the appearance of free serum antibody healing occurs. These temporal relationships indicate that the tissue lesions result from antigen-antibody combination occurring when antigen reacts with antibody formed fixed to tissue.

The Arthus reaction, which results from the combination of circulating antibody with antigen introduced into the skin, occurs after the appearance of free antibody at a time when tissue lesions are regressing.

Cortisone and serum sickness

The action of cortisone on experimental serum sickness is summarized in table 6. Rabbits given a single intravenous injection of 0.5 gm. of bovine albumin were sacrificed on the 12th day. One

Table 6

The effect of cortisone on the induced hypersensitivity of serum sickness in rabbits

Effect	Number showing effect	
	Control	Cortisone-Treated
Total number of animals	25	23
Antibody	7	5
Arthus reaction	5	3
Subendothelial fibrinogen	18	0
Necrotizing arteries	7	0
Endocarditis	11	1
Glomerulonephritis	20	4
Lesions		
Spleen	20	16
Lymph node	8/16	0/6

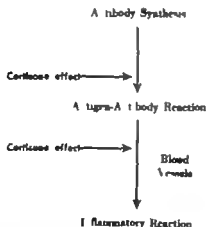


Figure 13. Possible roles of cortisone action in the allergic state.

half the group received a daily dose of cortisone, 5 mg. per kg. of body weight during this period. Cortisone did not inhibit antibody formation or the Arthus reaction under these circumstances, although the incidence and severity of visceral lesions were markedly

modified. Granulomatous lesions in the spleen of cortisone-treated rabbits were smaller and fewer in number. The mechanism by which cortisone inhibits visceral allergic lesions without suppression of antibody production is not clear from this experiment.

Cortisone may inhibit anaphylactic allergy by two distinct means, (1) suppression of antibody production, and (2) suppression of the damaging effects of antigen-antibody union. Many clinical cases achieving therapeutic benefits from cortisone seem to involve the latter mechanism while suppression of antibody production is not usually observed (figure 18).

The Role of Complement in Hypersensitivity

DR. FREDERIC C. MOLL. Since the fixation of complement is used daily in diagnostic laboratories as an index of antigen-antibody union, the functions of complement must also be better understood. However its instability, complex structure, and varying behavior under various conditions make description of its function difficult. The most consistent *in vivo* finding is the diminution in the level of circulating blood complement accompanying some pathological states ascribed to antigen-antibody interaction. Low complement titers in acute nephritis, rising to normal during convalescence, and low complement titers during serum sickness have been described. In the absence of hepatic disease the finding of low serum complement *in vivo* is regarded as an index of antigen-antibody reaction.

To delineate the relationship of complement to antibody formation, a group of rabbits were given a single large injection of heterologous serum, and serial antigen-antibody and complement studies were performed. Uninjected control animals show no change in serum complement level, whereas rabbits receiving bovine gamma globulin manifest a pattern typical of many antigens and of many other species. Eight to 10 days after injection the blood complement level falls, rising in two to four days, a time when circulating antibodies can be demonstrated. Animals treated with x-ray, nitrogen mustard or cortisone in doses large enough to suppress antibody formation did not manifest decrease in serum complement concentration.

Since pathological lesions attributable to hypersensitivity and

complement depression have been described only in animals given large doses of antigen, the relationship of quantity of antigen to antibody response requires consideration.

By the use of graded amounts of antigen it can be demonstrated that those animals receiving the smallest amount of antigen show less variation in serum complement and have fewer tissue lesions. Circulating antibody titers, however, are as high in those animals given the smaller amounts of antigen as in those given the usual large amounts. While there is apparently no correlation between the symptoms of serum sickness and the appearance of circulating antibodies in clinical medicine, experimental animals behave differently. The lesions of hypersensitivity can be demonstrated only when antibody is present.

Administration of ACTH prevents neither antibody formation nor the characteristic fall in serum complement, but does prevent the occurrence of lesions. Since complement level decreases, antigen-antibody interaction must occur with ACTH in some manner which protects the cell from damage. Cortisone administered to the rabbit prevents lesions by interference with antibody formation, as evidenced by failure to find precipitin in the circulating blood, and failure to observe any alteration in serum complement level.

During an acute episode of serum sickness, change in serum complement may be detected in many cases. The host reaction in general cannot be predicted except that it appears to be related to the amount of antigen administered. Anaphylactic states in man, typical in asthma, may occur without demonstrable change in serum complement titers.

Discussion

DR. THOMAS: Is there an effect of cortisone on the tissue distribution of antigen? Is there less or more antigen in the heart or kidney during cortisone treatment? What happens to animals given cortisone or ACTH for a short period, with subsequent antibody formation? The amount of foreign protein deposited in the tissues may be more critical than any change occurring in the circulation.

DR. DIXON: Serum protein antigens existing *in vivo* equilibrate with Π cells to which they have access. The concentration within tissues parallel that within circulating blood plasma, so that the blood level reflects tissue levels. We have never found selective retention or concentration of these antigens in any tissue.

DR. DOUGHERTY: The administration of horse serum to mice.

followed by the administration of ACTH or cortisone to suppress antibody formation followed by a period of no hormone administration, results in the rapid development of lesions in the heart and lungs, which differ from the usual serum lesions. Sloughing of bronchial epithelium without inflammatory signs is noted. A non-inflammatory myocarditis with degenerative changes of the myocardium is evident. Three or four days later an inflammatory phase sets in.

Relationship to Disease in Man

Collagen Diseases

DR. ENRICH The term collagen diseases designates a group of maladies characterized morphologically by systemic involvement of connective tissue. The concept of a systemic disease presupposes the presence of a biological system, and connective tissue is such a system. Made up of an association of fibroblasts and collagen fibres held together by an amorphous ground substance, connective tissue functions as supporting tissue. Interposed between the parenchymes and the blood and lymph vessels, it also serves as a site of transfer of metabolites and plays an important role in salt and water balance.

The recognized collagen diseases are rheumatic fever, rheumatoid arthritis, lupus erythematosus disseminatus, generalized scleroderma, dermatomyositis, serum sickness and polyarteritis. Glomerulonephritis is not included among these diseases because it is not a systemic disease of connective tissue. Since the heart and blood vessels, and sometimes other elements of the mesenchyme, are affected, it is better to define the collagen diseases morphologically as systemic diseases of the vascular-connective tissue.

If it is true, as postulated in this paper, that these diseases are dysgammaglobulinemias pathogenetically, the involvement of the vascular system is a condition *status quo a n* because the noxious agent, gamma globulin, has to pass through this system before it can contact the connective tissue.

Distinguishing characteristics

The collagen diseases are characterized clinically by their intermittent course and tendency to chronicity. Chemically they are distinguished by a depression of the serum albumin and of the collagenase inhibitor during acute stages, and by a rise in fibrinogen, alpha globulin, gamma globulin, glycoprotein, the C-reactive protein and the non-specific hyaluronidase inhibitor during acute

and subacute stages. Morphologically mucoid degeneration, fibrinoid degeneration and necrosis, granular formation and fibrosis (sclerosis) are common findings.

The chemical alterations observed in collagen disease appear to be unspecific manifestations indicative of liver and vascular connective tissue injury. The morphological changes represent an initial injury reaction to injury and repair. The rise in gamma globulin appears to play a key role in the pathogenesis of lesions characteristic of collagen disease. A specific reaction, the elevation in gamma globulin is not the result of tissue damage, but is rather due to plasma cell proliferation in the diseased connective tissue and bone marrow.

Etiology

A common etiologic origin for the collagen diseases cannot be found in their ultimate causation. Such states as serum sickness, polyarteritis, and rheumatic fever are wholly or partially allergic in nature with no evidence of an allergic state found for others. There is no sound basis for a belief that all collagen diseases are due to allergy. The concept that collagen diseases represent failure of adaptation with an increase in pituitary and adrenal cortical function, has not been confirmed.

The common denominator is apparently the hypergamma-globulinemia due to plasma cell proliferation. In serum sickness, polyarteritis, and rheumatic fever antibody becomes injurious when combined with specific antigen. In the pure pathological gamma globulin, the L. E. factor is active in the absence of exogenous antigen. This situation seems to exist in rheumatoid arthritis and may be found eventually in generalized scleroderma and dermatomyositis.

The cause of collagen disease appears to be the production of abnormal gamma globulin from plasma cells. These proteins circulate through the vascular bed, passing into vessel wall to become deposited there, and through them to the supporting connective tissue where localized sites of tissue injury can occur.

Autoantibodies in Hematologic Disorders

DR. ROBERT EVANS: Autoantibodies constitute the serologic abnormality that appears responsible in most cases of acquired

hemolytic anemia, of idiopathic thrombocytopenic purpura and some instances of unexplained neutropenia. Whether antigens in the affected cells are involved in the formation of antibody like agents is not established. Injection of red cells into rabbits with or without adjuvants or foreign proteins has been followed by evidence of auto-sensitization in some of the animals. Positive antiglobulin serum tests are obtained transiently in rabbits injected intraperitoneally with the animal's own blood.

Since red blood cells of any donor are hemolyzed when transfused into a patient with autoimmune hemolytic disease, the auto-antibodies of hemolytic anemia are considered to lack specificity. Recently the autoantibody of one patient with autoimmune hemolytic disease was shown to have a specificity for the α antigen. While this specificity does not prove that the α factor in the patient's cells was responsible for evoking the antibody initially it does provide supporting evidence.

Drug and virus induced disease

In thrombocytopenic purpura caused by Sedormid® a mechanism of antibody production with important implications for auto-antibody formation can be demonstrated. The sera of patients with Sedormid sensitivity lyse platelets *in vitro* in the presence of Sedormid. It is postulated that following ingestion of Sedormid a Sedormid platelet conjugate is formed which is antigenic. In some individuals this conjugate evokes antibody that lyses platelets only when they are combined with Sedormid. The hypothesis that foreign substances such as virus particles or medicament may form conjugates with cell proteins that provoke antibodies which then sensitize the cell is attractive, but unproven at present.

The observation that certain cases of acute hemolytic anemia are due to virus sensitization of the red blood cell, forming the bridge between cell surface and antiviral globulin, is as yet unconfirmed. In a patient with viral pneumonia and autoimmune hemolytic disease studied recently we failed to demonstrate the presence of virus by electron microscope photographs and egg embryo transfer technique.

Properties of autoantibodies

Although all autoantibodies active in autoimmune hemolytic disease are observed to sensitize red blood cells to normal body temperature and pH, they are found to vary in many other properties.

The variation in behavior of red blood cell autoantibodies is indicated by the many methods required for their demonstration. A few produce complement hemolysis, or agglutinate sensitized cells in saline at 37 C. Others agglutinate sensitized cells in a colloidal medium, but for most of them the antihuman globulin serum technique is required to demonstrate their presence on the patient cells.

In a study of 34 patients with autoimmune hemolytic disease (table 7) only one had a demonstrable hemolysin in the serum for his own group O cells and the other group O cells tested. Although heat inactivation of the patient serum prevented hemolysis of normal red blood cells incubated in the serum, these same cells

Table 7

Reactions in demonstrability of autoantibodies in 34 patients with autoimmune hemolytic disease

Hemolysis in serum disappearing with heat inactivation	1
Agglutination in serum active at 37 C. in saline	1
Agglutination on patient's cells reactive in colloidal media (30% beef albumin)	13
Agglutination on patient's cells active in antiglobulin serum	
Strongly positive	30
Weakly positive	2
Negative	

showed a positive antiglobulin serum test, indicating that sensitization by antibody had occurred in the absence of complement. The hemolytic property of this patient's serum indicated a qualitative difference in the nature of the autoantibody.

The red blood cells of two patients failed to agglutinate in antiglobulin serum. The red blood cells (one of these showing a negative antiglobulin serum test, were agglutinated when centrifuged in 30 percent beef albumin, indicating the presence of antibody. An antiglobulin serum prepared by injecting the patient serum into rabbits produced a positive antiglobulin serum test with the patient cells. It was concluded from these observations that the antiglobulin sera prepared by injecting normal human serum into rabbits did not contain antibody for the particular protein in the patient's serum responsible for sensitization of the patient's red blood cells.

In the second patient serological abnormalities were not demonstrable on the cells or in the serum by any technique. Normal

red blood cells transfused into this patient were destroyed at a rate equal to the rate of destruction of the patient's own cells, although isoantibodies as well as autoantibodies could be detected neither before nor long after the transfusion. Removal of a 250 gm. spleen that showed no evidence of phagocytosis or hypersequestration resulted in complete remission of the disease. The possibility exists that red blood cell destruction in this patient was caused by an autoimmune process even though a serologic abnormality was not demonstrable. An antiglobulin serum produced by injecting this patient's serum into rabbits did not result in a positive test.

In two other patients the agglutination of the patient's cells in antiglobulin sera was relatively weak. Attempts to prepare more specific antiglobulin sera by injecting sera from these patients into rabbits were not successful.

Table 8

Prozone phenomenon, comparison of the agglutination of the cells of patient E II by antiglobulin serum upon immed to centrifugation and upon centrifugation after a period of incubation

Dilutions of antiglobulin serum	Cell agglutination	
	Immediate centrifugation	Incubation $\frac{1}{2}$ hour
1:5	2	—
1:10	4	—
1:20	3	±
1:40	3	1
1:80	3	2
1:160	2	2
1:320	±	3
1:640	—	3
1:1280	—	1
1:2560	—	—

Variations in the antiglobulin serum reactions of other patients were observed that are of significance. The prozone phenomenon was encountered in the agglutination of the cells of one patient in any \times dilutions of antiglobulin serum. Prozoning was eliminated by immediate centrifugation of the cell antiglobulin serum suspension (table 8). Agglutination of one patient's cells in antiglobulin serum was almost completely reversed in all dilutions by incubation for one-half hour at room temperature (table 9). The

same red blood cells were still agglutinable in 30 percent beef albumin, indicating that sensitization persisted. Normal red blood cells sensitized in varying dilutions of the patient's serum exhibited the same reversal of agglutination on incubation. These observations

Table 9

Prozone phenomenon, the effect of immediate centrifugation and centrifugation after incubation at room temperature on the agglutination of the cells of patient J D in dilutions of several antihuman globulin rabbit serum of approximately equal potency

Dilutions of antihuman serum	Cell agglutination					
	Immediate centrifugation			Incubation ½ hour		
	Serum #1	#3	#4	Serum #1	#3	#4
1:8	—	4	1	—	±	—
1:10	2	2	2	—	±	±
1:20	2	2	1	—	—	—
1:40	3	1	—	±	—	—
1:80	4	1	—	±	—	—
1:160	2	1	—	—	—	—
1:320	2	—	—	—	—	—
1:640	2	—	—	—	—	—
1:1280	1	—	—	—	—	—
1:2560	—	—	—	—	—	—

indicate that the reversibility of agglutination was due to a property of the sensitizing agent, possibly an enzymatic action on the rabbit globulin.

Association-dissociation equilibrium

The presence of unattached autoantibody in serum is probably dependent on the amount produced and the degree of saturation of receptors on the red blood cells. There is evidence that dissociation of autoantibody from the cell takes place continuously indicating that an association-dissociation equilibrium exists. When sensitized cells are injected into the circulation of normal individual the recipient cells become agglutinable in antiglobulin serum, presumably due to a dissociation and transfer of autoantibody. It can be demonstrated *in vitro* that dissociation occurs continuously at 37 C without the destruction of the sensitized cells. When washed serial

tized group A cells are mixed and incubated for several hours with normal group B cells, and then separated by means of anti A sera, the group B cells can be shown to have acquired sensitization by their agglutinability in antiglobulin serum. Since there was no evidence of hemolysis during incubation it is apparent that destruction of the cell is not necessary for dissociation and transfer of autoantibody

Cold agglutinins

Four patients exhibited high serum titers of bivalent cold agglutinins that sensitized red blood cells and produced agglutination below body temperature, but were eluted from the cell at 37°C. Red blood cells collected from these patients at body temperature and maintained at 37°C during washing showed the usual evidence of sensitization with the antiglobulin serum technique. The sera of two patients produced sensitization of normal cells by a univalent antibody at 5 C, which did not become dissociated at 37°C during washing of the cells. The sera of the other two patients presented only the bivalent saline cold agglutinin. A univalent antibody cold or warm, could not be demonstrated by the antiglobulin serum technique nor with the use of trypanized cells.

Attempts to transfer the antibody found at body temperature from the cells of patients with cold agglutinins to normal cells were unsuccessful. Furthermore, sensitization of normal cells did not occur with eluates prepared from the patient's cells by various other techniques. In contrast, eluates made from cells of patients without high titers of cold agglutinins have shown a high degree of activity usually greater at 37 C than at lower temperatures. The failure to obtain active antibody in eluates from the patients with cold agglutinins points to a qualitative difference in the nature of the antibody protein.

Limitations of serologic methods

A feature of autoimmune hemolytic disease requiring explanation is the persistence of red blood cell sensitization during remission of the disease, spontaneous or induced by cortisone or splenectomy. The persistence of autoantibody on the cell indicates that the mere adherence of the plasma protein to the surface is not sufficient to bring about cell destruction.

Our fundamental understanding of the mechanism of red blood cell destruction in autoimmune hemolytic disease is dependent on quantitative studies of free and adsorbed antibody concentrations.

In general, a reduction in concentration of adsorbed antibody accompanies cessation in abnormal red blood cell destruction.

Serologic methods of limited accuracy are the currently available tools for quantitative study. It is probable that critical changes in concentration of adsorbed antibody occur without being reflected in agglutinability in dilutions of antiglobulin serum (table 10). The activity of eluates prepared from samples agglutinating at 1:320 titer varies markedly.

Table 10

Serologic quantitation of adsorbed antibody

Dilution of Anti-D serum	Highest titer antiglobulin serum producing agglutination	Agglutination of D cells exposed to dilutions of eluate from sensitized cells, by antiglobulin serum
		1:1 1:2 1:4 1:8 1:16
1:5	1:320	4 4 3 1 —
1:50	1:320	2 + — —

The results indicate that cells with identical agglutinability in high dilutions of antiglobulin serum may have varying quantities of adsorbed antibody as judged by the amount of antibody demonstrable in an eluate. 0.5 cc. of group O CDE cells are sensitized in 2 cc. volumes of Anti-D serum varying in concentration. After determining their agglutinability in antiglobulin serum dilutions, eluates were prepared by heating 0.5 cc. of the cells in 2 cc. of saline. Normal CDE cells are then exposed to serial dilutions of the eluate and the agglutinability tested by agglutination in 1:5 dilutions of antiglobulin serum.

The relationships of antibody adsorbed on cell surfaces to the active and quiescent stages of autoimmune hemolytic disease are shown in tables 11, 12, 13. With spontaneous remission the agglutinability of the cells diminishes. Cortisone therapy suppressing the formation of autoantibody produces a reduction not only in free serum antibody but in agglutinability of red blood cells. Splenectomy in these patients was followed by remissions of variable duration. Three of these patients did not manifest a significant change in the direct antiglobulin serum test even though splenectomy resulted in remission.

Idiopathic thrombocytopenic purpura

Most cases of idiopathic thrombocytopenic purpura are due to abnormality of plasma proteins similar to that seen in autoimmune hemolytic disease. More than one half the living infants born to

mothers with idiopathic thrombocytopenic purpura show transient thrombocytopenic purpura. A platelet-depressing factor has been demonstrated in 16 of 26 patients with idiopathic thrombocytopenic purpura, and the frequency of demonstration of platelet agglutination has varied from 28 to 66 percent in the series studied to date.

One disadvantage in the study of serologic abnormalities in idiopathic thrombocytopenic purpura is the necessity of demonstrat

Table II

Antibody concentration determined by direct antiglobulin serum dilution tests in autoimmune hemolytic disease during its active state and after spontaneous remission

Pt.	Sex	Age	Active Disease		Remission		Remarks
			Low	High	Low	High	
J.D.	M	32	1:160 1:160	1:1280 1:1280	0	1:80	Spontaneous remission followed by relapse
W.G.	M	31	1:160	1:320	1:1 1:160	1:10	Active hemolysis did not return
S.G.	M	42	1:50	1:640	1:10	1:640	Autoimmune hemolytic disease accompanying viral pneumonia
H.K.	M	42	1:200	1:400	1:10	1:50	Autoimmune hemolytic disease accompanying viral pneumonia

ing the immune agent in plasma. Free serum antibody may exist in low concentrations in some patients, as is the case in many with autoimmune hemolytic disease. Because of the difficulty in obtaining stable platelet suspension after the washing required, the use of the direct antiglobulin serum test was not successful. Although patients with acute and chronic thrombocytopenic purpura rapidly receive transfused platelets from the circulation, the disappearance of transfused platelets in idiopathic thrombocytopenic purpura needs re-evaluation. It has been postulated that isoantibodies formed as the result of previous transfusions may reduce the survival time

of platelets. Platelet agglutinins, like red blood cell autoantibodies, are not always panagglutinins, since the serum of one patient may agglutinate autologous platelets, but not agglutinate all other platelets tested.

Table 12

Antibody concentration determined by direct antiglobulin serum tests in autoimmune hemolytic disease during its active state and after remission following cortisone therapy

Pt.	Sex	Age	Active Disease		Remission		Remarks
			Low	High	Low	High	
D.H.	F	14	1400	1400	0	1-200	Ceptsote remission with negative direct antiglobulin test
L.P.	M	32	1320 1160	1640 11200	1-5	1-40	Quiescent state relapses with reduction of cortisone
H.B.	F	65	1400 1200	1800	1-50	1-50	Remission with relapse following withdrawal
L.M.	M	33	1640	1640	1160	1160	Free serum antibody disappeared during partial remission
W.H.	M	37	13200	16400	1400	11600	Partial remission prior to splenectomy
C.S.	F	76	16400	112000	1200	1400	Partial remission prior to splenectomy

Neutropenia and lupus

The probability that some instances of unexplained neutropenia, particularly those responding to penicillin, are due to autoantibodies still rests largely on the circumstantial evidence of association.

Evidence is accumulating that factors in the serum of patients with disseminated lupus erythematosus, responsible for the lupus

cell and for clumping of leucocytes in L. E. preparations, belongs to the category of abnormalities of plasma proteins referred to as autoantibodies. Other studies have shown that antileucocytes produce changes in leucocytes making them indistinguishable from

Table III

Antibody concentrations determined by direct antiglobulin serum dilution tests: autoimmune hemolytic disease during its active state and after remission following splenectomy

Pt.	Sex	Age	Active Disease		Remission		Remarks
			Low	High	Low	High	
G.T.	F	64	1:160	1:320	1:10	1:160	Remission
J.Z.	F	56	1:160	1:640	1:10	1:80	Remission
L.P.	M	22	1:160	1:1280	1:40	1:640	Remission
O.P.	M	32	1:80	1:160	1:20	1:160	Transient relapse following splenectomy
F.H.	M	58	1:160 1:320	1:640 1:640	1:5	1:80	Relapse 3 mos. after splenectomy
J.D.	M	52	1:160 1:320	1:1280 1:320	1:20	1:320	Remission; relapse after 1 year
W.H.	M	37	1:800	1:3200	1:400	1:1600	Remission 2 yrs.
H.R.	F	49	1:320	1:640	1:320	1:640	ACTH given following splenectomy
L.T.	F	67	1:800	1:6400	1:800	1:1600	Remission 3 mos.

I pus cells. Agglutinins for platelets, an autoantibody for red blood cells, and a factor producing the lupus cell have all been demonstrated in patient with lupus erythematosus with hemolytic anemia and thrombocytopenic purpura. The protein abnormalities responsible for these phenomena appeared to be separate and distinct, since the red cell-sensitizing agent eluted from preparations of red cell stroma, while active in sensitization of normal red cells, did not produce agglutination of platelets or the I pus cell changes in leucocytes.

The Metabolism of Mucopolysaccharides

DR. ALBERT DORFMAN In hypersensitivity states and related diseases, profound alterations occur in the ground substance of connective tissue. The chemical and physicochemical nature of these changes is almost entirely unknown despite widespread speculations based on histochemical studies.

Ground substance contains at least two acid mucopolysaccharides, hyaluronic acid and chondroitin sulfuric acid. The known chemical and physicochemical properties of these polyelectrolytes suggest that they may play an important part in the physiologic and pathologic behavior of connective tissue although the lack of specific data regarding their biochemistry makes an analysis of their functional potentialities impossible.

Isolation of hyaluronic acid

These studies have been carried out in collaboration with Roseman. As an experimental tool, Group A hemolytic streptococci afford an unusual opportunity for the study of the biosynthesis of hyaluronic acid, since biochemical experience has indicated that the pathways of metabolism in microorganisms bear close resemblance to those of mammalian tissue. The unique presence of hyaluronic acid in the capsule of the streptococcus, which is apparently chemically similar if not identical to that of connective tissue, is of particular interest in view of the peculiar character of the reactions of the human to streptococcal infection.

A strain of Group A hemolytic streptococcus was cultured in a semisynthetic medium, and methods were devised for the isolation of hyaluronic acid from this culture. The high degree of purity of this preparation was established by both multiple analyses and electrophoresis. In the analytical results shown in table 14, the somewhat low values for uronic acid, as evidenced both by uronic CO_2 analysis and neutralization equivalent, are apparent. Similar discrepancies are present in the original data of Kendall and Heidelberger and may be of some significance in indicating chemical differences between streptococcal and connective tissue hyaluronic acid. If such differences exist they may open up new possibilities with respect to the effects of streptococcal products on mammalian tissues.

The hyaluronic acid isolated from the culture supernatant was subjected to degradation procedures which made possible the de-

termination of the location of radioactive carbon in the various parts of the molecule.

Table 14

Analysis of hyaluronic acid

Analysis	Method	Observed	Theory	Observed
				Theory
		percent	percent	
C	Microcombustion	44.37	44.33	1.00
H		5.93	5.58	1.06
N	Kjeldahl Nessler Dumas	3.7 ^a 3.55	3.69	1.01 .96
Uronic CO ₂	Gaseometric Titrimetric	10.31 10.60	11.60	.89 .91
N Acetyl	Chromic Acid	11.74	11.35	1.03
Neutralization equivalent	Titrimetric	355	379	.94
Ash S P		—	—	
Glycogen	I ₂	—		
H ₂ Iaronidase	Turbidimetric	+++		
[α]		-67.5		

All analyses performed on dried samples or corrected for moisture content
^aBased upon (C₁₂H₁₀O₆N₂)

Hyaluronic acid precursors

To establish the precursors of hyaluronic acid, various compounds, into which radioactive carbon had been introduced in specific positions, were incorporated into a semisynthetic medium upon which a strain of streptococcus was grown. When 1-C¹⁴-glucose was incorporated into the growth medium with a radioactivity of 30,858 c.p.m. (measured as BaCO₃ at infinite thickness) pure hy

aluronic acid was isolated with an average radioactivity of the 14 carbon atoms of hyaluronic acid of 5,231 c.p.m.

After hydrolysis of the polysaccharide, glucosamine, acetic acid, and carbon dioxide produced by the decarboxylation of uronic acid were isolated. A portion of the glucosamine was oxidized to glucosaminic acid, and the latter decarboxylated with ninhydrin to yield carbon dioxide, which represents the first carbon atom of the glucosamine. From the residue of the decarboxylation reaction rabinose was isolated, giving a measure of the radioactivity of the C₅₋₆ of the glucosamine molecule. In addition to these components acetic and lactic acids were isolated from the medium as benzimidazole derivatives.

The radioactivity of the first carbon atom of the glucosamine was very similar to that of the glucose in the medium, indicating that the glucosamine portion of the hyaluronic acid molecule rises from the glucose without previous scission of the glucose molecule. The radioactivity of the acetyl portion of the molecule was found to be identical with that of the acetate of the medium, suggesting that the two either rise from the same precursors or are in equilibrium. The radioactivity of the sixth carbon atom of the uronic acid was found to be lower than was that of C₅₋₆ of the glucosamine, indicating that under the conditions of these experiments little resynthesis of glucose from glycolytic products occurs. When the total amount of radioactivity of the hyaluronic acid was compared with that accounted for by the parts that were isolated and degraded, there was a discrepancy of 29,678 counts. Since only the first five carbon atoms of the uronic acid were not isolated, it follows that this amount of radioactivity must reside in these carbon atoms. This could be readily accounted for by activity in the first carbon atom comparable to that of the first carbon atom of the original glucose, but was not established with certainty in this experiment. Because the isolation and degradation of the uronic acid portion of the molecule presents considerable experimental difficulty a simpler method was sought. Accordingly 6-C¹⁴-glucose was synthesized and incorporated into the medium. The radioactivity of the sixth carbon atom of the uronic acid (which is readily obtained by decarboxylation) was found comparable to that of the original glucose suggesting that the uronic acid portion of the hyaluronic acid is formed from glucose without previous scission of the molecule. The C₆ of the glucosamine was isolated and showed comparable radioactivity of rabinose confirming regarding the origin of the hexosamine.

With the remote origin of the fourteen carbon atoms of hyaluronic acid established, recent experiments have been aimed at the elucidation of the more immediate precursors. Utilizing glucosamine labeled with C^{14} in the one position and N^{15} in the amino group we sought to determine whether glucosamine as such is incorporated into the polysaccharides. The doubly labeled glucosamine was prepared biologically by growing *Aspergillus niger* in the presence of 1- C^{14} -glucose and $N^{15}H_4NO_3$. Labeled glucosamine was isolated from the chitin of the mycelium and incorporated into the medium for streptococci. The remainder of the experiment was then conducted as previously discussed. The finding of a constant C^{14}/N^{15} ratio indicates that glucosamine is used directly in hyaluronic acid synthesis.

To determine whether glucuronic acid was incorporated as such, 6- C^{14} -glucuronolactone was synthesized and utilized as an intermediate. The isolation of the sixth carbon atom of the glucuronic acid portion of the hyaluronic acid indicated no incorporation of the glucuronolactone. Since the lack of incorporation of glucuronic acid might be due to its glycolysis prior to hyaluronic acid synthesis, other organic acids were isolated from the medium. These showed no radioactivity suggesting that glucuronic acid is not metabolized via this pathway. This failure to utilize uronic acid may be due to impermeability of the cell, or may indicate that oxidation of the sixth carbon atom occurs only after glycoside formation.

In subsequent studies utilizing carboxyl-labeled acetic acid and carboxyl-labeled N-acetyl glucosamine we have demonstrated that acetic acid can serve as precursor of the N-acetyl group of hyaluronic acid, and that a deacetylase exists that can hydrolyze the acetyl group of N-acetyl glucosamine. The evidence obtained thus far suggests that N-acetylglucosamine is not utilized for hyaluronic acid synthesis.

Mucopolysaccharide metabolism in skin

After development of method for the isolation of hyaluronic acid and chondroitin sulfuric acid from skin, a study of mucopolysaccharide metabolism in mammalian skin was instituted. These studies have been carried out in collaboration with Schiller. Defatted ground skin was extracted with a 0.2 percent solution of sodium hydroxide. Following dialysis and adjustment of pH the extract was treated with trypsin and further dialyzed. Proteins were precipitated with trichloroacetic acid and the polysaccharide precipitated

from the supernatant with alcohol. The crude mixture of polysaccharides so isolated was separated by zone electrophoresis on celite.

Two tenths of a millimole of carboxyl labeled acetate was given to rabbits subcutaneously daily for periods up to eight days. Following this the animals were sacrificed and polysaccharides were isolated from the skin. A greater rate of synthesis of hyaluronic acid than chondroitinsulfuric acid was apparent. These same results were obtained whenever 1-C¹⁴-glucose was administered.

Subsequent experiments studying the rate of disappearance of hyaluronic acid and chondroitinsulfuric acid from skin have confirmed these conclusions². Hyaluronic acid has a half life time of between two and three days in rabbit skin while chondroitin has a half life time of about eight days. Experiments utilizing C labeled acetate C¹ labeled glucose, and S³⁵O₄ suggest that the rate measured represents complete breakdown and resynthesis of the polysaccharide molecule.

Hyaluronidase Inhibitor and Heparin in Human Blood Serum

DR. DAVID GLICK Studies on the nature and role of the non-specific hyaluronidase inhibitor existing in human blood serum, possibly employed as a defensive agent by higher animals against bacterial invasion, have been in progress for several years. It has been established that higher forms of life possess at least two mechanisms for the inhibition of the hyaluronidase elaborated by an invading organism. The integrity of defensive intercellular walls may depend on these mechanisms.

The production of specific antibody in response to a specific enzyme elaborated by an invading species constitutes one of these mechanisms. Since this method of defense involves a time factor for production of antibody, general and more immediate available defense factors would seem desirable to inhibit the activity of hyaluronidase from any source.

Heparin and hyaluronidase inhibitor

A relationship between heparin and such a nonspecific hyaluronidase inhibitor is suggested from findings that the serum concentrations of both are elevated during periods of stress, such as in

bution usually tends to be of the all or none variety. For quantitative studies it is necessary to use connective tissue membranes *in vitro* such as rabbit urinary bladders, or intact membranes *in vivo*. For the latter the talocrural articulation of the rabbit was selected. This joint, consisting of a small compartment lined with a thin membrane, is less complex than the knee joint.

Table II

The influence of various substances on the permeability of the ground substance

Enhancement	Inhibition
Hyaluronidase*	Cartilage
Lysozyme	Adrenal Cortical Extract
Desoxyribonuclease	Estrogen
Ascorbic Acid	Testosterone
Dehydro-ascorbic Acid Spreading factor from human urine	Salicylates

*Determined by the spreading reaction

While the spreading reaction is independent of local circulatory phenomena, *in vivo* methods that involve clearances may not be. The qualitative results, however, are identical with those obtained with isolated membranes with spreading tests.

Synovial membrane permeability

The anionic dyestuff PSP (phenolsulfophthalein) injected into the talocrural articulation of the rabbit is rapidly absorbed and excreted in the urine. This pattern of rapid clearance of PSP is altered by variety of factors. Hyaluronidase injected into the synovial cavity along with the dye produces an increase in the rate of dyestuff elimination. The same rapid disappearance of PSP from the synovial cavity is observed in nephrectomized rabbits. However the injection of hyaluronidase into the synovial cavity does not increase the clearance of intravenously administered PSP.

Intramuscular desoxycorticosterone (1 mg per kg. of body

weight) administered before the dye is injected, acts similarly to hyaluronidase. These drugs together do not produce an enhanced effect. An increase in permeability is observed after the intra-articular injection of lysozyme or deoxyribonuclease, or massage of the joint.

Cortisone or ACTH administered before the dyestuff completely inhibits synovial membrane permeability. Hyaluronidase does not antagonize the cortisone effect. Estrone, testosterone, and ascorbic acid decrease the permeability to a lesser degree than does cortisone.

Adrenalectomized rabbits show an inhibition of the normal spreading reaction. Hyaluronidase enhances this reaction proportionate to the amount of enzyme administered, and is independent of the presence or absence of adrenal glands. Adrenalectomy in rabbits decreases the permeability of the synovial membrane *in vivo*. Hyaluronidase is effective in increasing the membrane permeability of adrenalectomized rabbits. Cortisone completely blocks membrane permeability while ACTH is without effect in the adrenalectomized animal.

These data indicate that the permeability of the ground substance is markedly influenced by the presence of the adrenals, and most agents which depress permeability do so through an adrenal mechanism. Few substances have a direct effect.

The accumulation of hyaluronic acid in some tissues during thyroid dysfunction and the antipermeability effect of estrone and testosterone indicate that the ground substance is affected by the thyroid and gonads as well as by the adrenals.

Since these studies demonstrate that the ground substance is markedly influenced by several physiologically occurring substances, it is not necessary to invoke the presence of hyaluronidase in tissues as a normal physiologic agent. Although it is not possible to demonstrate hyaluronidase in tissues outside the sperm, current assay methods may not detect concentrations of the enzyme that might be active elsewhere.

Permeability in experimental serum disease

To determine whether the beneficial effects of adrenal stimulation in nephrotoxic serum disease in the rabbit is due to suppression of lymphoid tissue alteration, permeability of ground substance to antigen-antibody comparison was made of the effects of cortisone, 21-acetoxypregnenolone, desoxycorticosterone and hyaluronidase in experimental serum disease.

Cortisone affects the ground substance as well as lymphoid

tissue while 21-acetoxypregnenolone stimulates the adrenals to release an antipermeability factor and has little effect on lymphoid tissue. Hyaluronidase enhances the permeability only of the ground substance. Cortisone was found to prevent the cardiovascular lesions of nephrototic serum disease. This protection could be due only in part to antipermeability action because 21 acetoxypregnenolone given in dose calculated to equal the antipermeability effect of cortisone was without influence on the lesions.

Hyaluronidase markedly increases the incidence and severity of lesions in the heart and blood vessels. These observations indicate the pronounced influence of permeability in the course of a sensitivity disease.

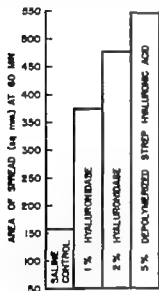


Figure 14 Activity of depolymerized hyaluronic acid as spreading factor relative to hyaluronidase.

Peptizing property of hyaluronate

The subcutaneous injection of rabbits with hyaluronidase results in an increase of hyaluronate-like staining material in the distal tubule and a discharge of hyaluronate into the tubular lumen. The subcutaneous injection of hyaluronidase in patients with renal calculi excreting turbid urine is followed by the excretion of clear urine. Utilizing *in vitro* methods it can be demonstrated that hyaluronate is a peptizing agent and protective colloid.

If the effect of hyaluronidase on urine is the result of release and activation of hyaluronic acid, then stimulation of the adrenals

or the injection of cortisone should have the reverse effect. Subjecting a healthy adult to stress, injection of cortisone, or injection of ACTH leads to the production of turbid urine which is not due to phosphaturia or change in urinary pH. The urinary excretion of glucuronides, an indirect measure of hyaluronic acid, is decreased.

The fact that hyaluronic acid present in the circulation finds its way into the urine suggests the possibility that hyaluronic acid itself may be an active spreading factor if present in the proper molecular form. Depolymerized streptococcal hyaluronic acid has been found to be an active spreading factor as hyaluronidase (figure 14)

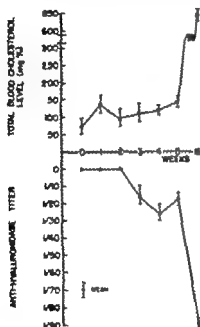


Figure 15. Relationship between serum cholesterol and anti-hyaluronidase titer in rabbits fed high cholesterol diet and injected with hyaluronidase.

Hyaluronidase in hypercholesterolemia

Hypercholesterolemia experimentally produced in rabbits may be controlled for a limited time by the subcutaneous administration of hyaluronidase. Concurrent with the production of an anti-hyaluronidase, an abrupt increase in blood cholesterol is observed (figure 15). The period of protection afforded by hyaluronidase does not spare the rabbits from the pathologic changes associated

with hypercholesterolemia. Greater uptake of cholesterol by endothelial cells was apparent in the treated rabbits, which was consistent with the higher degree of dispersion of serum lipids noted on dark field microscopy. Endogenous hypercholesterolemia as seen in rats with nephrotoxic serum disease is also controlled by the administration of hyaluronidase or hyaluronic acid.

The administration of large doses of hyaluronic acid, isolated from a highly virulent strain of streptococcus, in acute toxicity studies resulted in a mortality of one in 250 animals. There were no histopathologic changes in the tissues of this animal. The pathologic manifestations observed during infections with this type of organism are therefore not likely due to the hyaluronic acid such organisms might release.

Much remains to be learned of the normal physiology of the ground substance, the changes that occur in it during pathologic states, and the way in which drugs achieve therapeutic results in disease.

Discussion

CHAIRMAN ARTHUR L. BLOOMFIELD: In light of what was said regarding the inhibition of the immune reaction by steroid, would you say such therapy should be discontinued at the first symptom of infection, viral or bacterial, or is it too late then?

DR. RAFFEL: Answering this from the experimental standpoint, such therapy should be discontinued for reasons of infection. I don't think that the allergic reactivity which accompanies most infectious processes is such as to do obvious early injury to the host from the standpoint purely of allergic destructiveness. Hence there is no good basis for providing steroids simply to depress this phase of the host response. And since these substances seem to encourage the infectious process it would seem to be a good idea to discontinue them.

CHAIRMAN BLOOMFIELD: Since decrease in eosinophiles follows administration of ACTH or cortisone, is there any evidence that they are the carriers of either antigen or antibody?

DR. DOUGHERTY: The best evidence at present is that intravenous cortical hormone does not increase sequestration of eosinophiles in the spleen, bone marrow or lungs. Thus eosinopenia is not due to a shunting out of eosinophiles into these areas. The eosinopenia is most likely due to lytic action of the hormone which produces a degranulation of the cell that is very important from clinical standpoint for those who use the eosinophile count, since the number of granules that remain determine whether you

classify the cell and eosinophile or not. This is probably one of the reasons for the discrepancies found in eosinophile counts. There is work suggesting that antigen is contained in eosinophiles. Guinea pig eosinophiles given passively to other guinea pigs immunized to the homologous antigen may shock. This can be prevented by heparin.

CHAIRMAN BLOOMFIELD Is there any evidence that steroid therapy either inhibits or accelerates the formation of histamine from the antigen-antibody complex?

DR. GOOD The only evidence I know about is the evidence that antibody complexes cannot inhibit a histamine reaction.

CHAIRMAN BLOOMFIELD Are we any closer to understanding the role of the thymus gland?

DR. THOMAS No.

CHAIRMAN BLOOMFIELD Can concentrated human gamma globulin be in any way considered nonspecific?

DR. THOMAS Yes.

CHAIRMAN BLOOMFIELD Why does the passive immunity of the newborn to rubeola and poliomyelitis last for six to nine months? Is it purely a quantitative matter?

DR. DIXON I think it probably is. The duration of passive immunity would be determined by the rate of catabolism of antibody in early childhood. Antibody decreasing with a half life of three or four weeks might well cover the infant for that period of time.

DR. DORFMAN There is no simple chemical mechanism or reasonable metabolic pathways by which a mucoprotein can arise through destruction of mucopolysaccharide. Thus, relating a rise in serum mucoprotein to a change in connective tissue mucopolysaccharide is without reasonable basis.

There are many theoretical reasons why one should be dubious about the role of hyaluronidase and hyaluronidase inhibitors in such disease states as rheumatic fever. Hyaluronidase may play no more role in the breakdown of hyaluronic acid than does amylase in glycogen degradation, synthesis and storage. The biochemistry of ground substance is extremely complex and we know little about it at present.

DR. ENRICH: If hyaluronidase inhibitor is heparin, its action in preventing fibrinoid and fibrin formation in disease may well be interpreted in teleological terms in the natural defense mechanism.

DR. GLICK Teleology has its uses primarily in serving as a guide. It can give direction to experimental design. It also has its weaknesses and is not to be accepted uncritically. Teleology has

been compared to a woman with whom you like to keep company but with whom you don't want to be seen on the street.

I don't think it is entirely accidental that nature has endowed many predators with enzyme hyaluronidase. Many pathogenic organisms possess this enzyme and in nature every object of prey has some kind of defense to the predator. It is not unreasonable to assume that hyaluronidase inhibitor plays a defensive role against the invasive factor but this is offered as a possibility not as a fact.

CHAIRMAN BLOOMFIELD: A clinician who sees rheumatic fever might ask this question. You have first a hemolytic streptococcus infection of the throat, tonsillitis, which subsides. There is no more hyaluronidase getting into the blood stream after the acute phase and still there is a latent period of three weeks longer before the onset of rheumatic fever. How do you fit hyaluronidase into this?

DR. GLICK: My guess would be that the invading organism is highly adaptive. It has been shown by *in vitro* experiments that organisms grown in a hyaluronic acid-rich medium increase their hyaluronidase. The latent period might involve a development of the enzyme to increase invasiveness of the streptococcus to a critical level.

DR. EHRLICH: There may be a simpler explanation. The streptococci that produce rheumatic fever are those which form a capsule of hyaluronic acid. This capsule interferes with phagocytosis. Hence, the bacteria will live longer in tissues and thus elicit more antibody in the host. It has been noted that those patients developing rheumatic fever are those that produce higher antibody titers than others. I think it is the hyaluronic acid nature of the capsule rather than the hyaluronidase which determine the end result. The two types of streptococci which are the best producers of hyaluronidase (Types 4 and 22) are never observed in connection with rheumatic fever.

DR. DORFMAN: The properties of hyaluronidase inhibitor are chemically very different from heparin. If heparin plays a role it can only be as part of a complex, and the direct presence of heparin and pre-hyaluronidase inhibitor has not been demonstrated. We have recently been able to isolate the blood hyaluronidase inhibitor in pure form and have been unable to find evidence of the presence of heparin.

DR. DOWNIE: In regard to the question of persistence of antibody formation in the absence of antigen it may be very difficult to be sure that there is antigen present. I agree with Dr. Dixon that there is a good deal of evidence to suggest that antibody formation

may go on in the absence of antigen after it has been eliminated from the tissue. If plasma cells are concerned with antibody formation I should like to ask what is the life span of plasma cells, and if they live a short time do they divide to other cells which continue to form antibody? Does antigen influence the cell so that the property of forming antibody may be passed on to those cells?